

THE CHEMISTRY OF NATURAL PRODUCTS RELATED TO PHENANTHRENE

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SECOND EDITION

With an Appendix

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BOOK DEPARTMENT

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GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in cooperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, Secretary of the American Chemical Society, Washington, D. C.; John E. Teeple, Treasurer of the American Chemical Society, New York City; and Professor Gellert Alleman of Swarthmore College. The Trustees have arranged for the publication of the American Chemical Society series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company (Reinhold Publishing Corporation, successors) of New York City.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed the editors, named at the close of this introduction, to have charge of securing authors, and of considering critically the manuscripts prepared. The editors of each series will endeavor to select topics which are of current interest and authors who are recognized as authorities in their respective fields. The list of monographs thus far secured appears in the publisher's own announcement elsewhere in this volume.

The development of knowledge in all branches of science, and especially in chemistry, has been so rapid during the last fifty years and the fields covered by this development have been so varied that it is diffi-

cult for any individual to keep in touch with the progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and such compendia as Beilstein's *Handbuch der Organischen Chemie*, Richter's *Lexikon*, Ostwald's *Lehrbuch der Allgemeinen Chemie*, Abegg's and Gmelin-Kraut's *Handbuch der Anorganischen Chemie* and the English and French Dictionaries of Chemistry, it often takes a great deal of time to coordinate the knowledge available upon a single topic. Consequently when men who have spent years in the study of important subjects are willing to coordinate their knowledge and present it in concise, readable form, they perform a service of the highest value to their fellow chemists.

It was with a clear recognition of the usefulness of reviews of this character that a Committee of the American Chemical Society recommended the publication of the two series of monographs under the auspices of the society.

Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfillment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well-digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, it is intended to include extended references to the literature, which will enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection will be made of those papers which are most important.

The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a serious attempt to found an American chemical literature without primary regard to commercial considerations. The success of the venture will depend in large

part upon the measure of cooperation which can be secured in the preparation of books dealing adequately with topics of general interest; it is earnestly hoped, therefore, that every member of the various organizations in the chemical and allied industries will recognize the importance of the enterprise and take sufficient interest to justify it.

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PREFACE TO THE FIRST EDITION

The six-year period since 1929 has witnessed the expansion of the previous, small list of naturally occurring phenanthrene compounds to include several groups of substances which are as strikingly similar in structure as they are different in their actions on the animal organism. The century-old problem of the structure of the sterols and bile acids reached a culmination in 1932 with the recognition of the presence in the molecules of the characteristic perhydrocyclopentenophenanthrene ring system, and it was not long before the recently discovered sex hormones were found to be similarly constituted. In rapid succession the cardiac glycosides, the heart poisons secreted by toads, and certain of the hemolytic saponins were revealed as cyclopentenophenanthrene derivatives. It is now known that sterols are synthesized in the organisms of higher animals as well as in plants and it appears likely that they are the natural precursors of all of the substances of related structure, yielding bile acids, male and female sex hormones, heart poisons, saponins, and antineoplastic agents as products of biological oxidations and reductions.

Also characterized as hydrophenanthrene derivatives are the alkaloids of the morphine and aporphine groups, the acids obtainable from resins of conifers, and the triterpenoid saponins. Although there are no indications of any connection in plant biology between these substances and the sterols, there are many interesting correlations in the properties and chemical reactions of all of these phenanthrene derivatives and in the history of the determination of their structures. Synthetic methods developed for the purpose of identifying aromatic degradation products in one series have frequently been applied to distinct advantage in attacking the other problems. This synthetic work establishes a direct connection to the chemistry of the cancer-producing hydrocarbons, for nearly all of these remarkable substances contain the phenanthrene nucleus. Furthermore, the most potent carcinogenic hydrocarbon yet known was first obtained as a degradation product of a bile acid. There is some possibility that hydrocarbons of this type may be formed in the body, and there are some suggestions of a possible relationship to the oestrogenic hormones, but regardless of what the ultimate decision may be on these important points the tumor-producing hydrocarbons are currently of considerable interest in connection with the chemistry and the physiological actions of the phenanthrene compounds recognized as true products of biosynthesis.

The preparation of the present volume was undertaken with the idea that a review covering all of these topics in phenanthrene chemistry might be of value in consolidating the advances already made and in expediting the further development of the field. All known types of phenanthrene compounds have been considered in the discussions whether they occur as such in nature or are formed as secondary transformation products, and the chemistry of phenanthrene itself is presented in an introductory section in order to provide a background for the consideration of derivatives which are of interest either as degradation products or as intermediates in synthesis. It has been the aim to present a comprehensive survey of the more significant and useful observations in each of the separate fields and to give prominence to correlating principles and to other matters of central interest. The history of the masterful work of elaborating the structural formulas is highly interesting and instructive, and an effort has been made to capture something of the spirit of the investigations and some essence of the truly dramatic disclosures of the past six years. The structures of key compounds of all of the groups are now either fully established or known in the most essential details and there are indications that the chemical work is entering the phase of total synthesis. It is perhaps appropriate to emphasize the known processes for the construction of the phenanthrene ring system because the methods which heretofore have been employed chiefly in identifying products of degradation may be of service in attacking the much more difficult problem of synthesizing the natural products themselves.

In the case of the older problems it appeared profitless to discuss in any detail the early literature dealing in some cases with the isolation of a profusion of "compounds" of questionable individuality. The isolation of the sex hormones, on the other hand, is an achievement of strictly modern technique, and the work is of considerable current interest not only because it exemplifies the practical value of micromethods but also on account of the biological implications associated with the occurrence of the hormones and their companion substances. Few conscious omissions have been made in describing these important new developments. In treating such borderline subjects as that of the sex hormones, the point of view is that of a chemist interested in learning the main facts and lines of evidence regarding the physiological functions of the compounds in question, but not wishing to venture too far beyond his own field in the matter of detail. The morphine problem, except for the work on drug addiction, has been given somewhat less prominence than the other topics simply because the major advances belong to an earlier period and are better known.

In coping with the formidable question of nomenclature and number-

ing the principle has been to follow precedent where this is sufficiently clear, and in other cases to adopt the system which appears best suited to present and future needs without preferential regard to any one of the current usages. In the hope of clarifying the nomenclature of the sterols without introducing radical changes, the prefixes *epi-* and *allo-* have been italicized only when they refer to the configurations at the 3- and 5-positions, respectively. As for the formulas, it may be noted that the use of wax engravings has afforded a convenient means of expressing definite theoretical views regarding the bond structures of the polynuclear hydrocarbons. In the case of phenanthrene, for example, the disposition of the shorter of the two lines representing a double bond is such as to indicate that the aromatic unsaturated linkages between the central nucleus and the terminal rings are not equally shared but belong principally to the terminal rings, leaving the 9,10-double bond in a comparatively isolated condition. In representing anthracene and other compounds of quinonoid structure it is considered that an ethylenic linkage held between a quinonoid and a benzenoid ring is claimed principally by the latter structural unit. The bond structures assigned to unsymmetrical derivatives and benzologues of anthracene are based upon the principles governing the equilibria in tautomeric quinonoid systems, essentially as defined by Kehlmann in 1898. 1,2-Benzanthracene, for example, is considered to exist chiefly in the β -naphthoquinonoid, rather than the α -benzoquinonoid, form because of the greater thermodynamic stability of the corresponding quinone of the former type. Attention is called to these views because, although they are not discussed in the body of the book, they are intentionally implied in the formulas.

In a different category are certain suggestions as to the possible structures of some of the less completely characterized natural compounds or their transformation products (ouabain, scillaren A, digoxigenin, ergosterol peroxide, the sterol-like sapogenins, certain toad poisons). Although these ideas admittedly are speculative, and although the suggestions may serve only to prompt their experimental refutation, the inherent difficulty of the problems of structure and the rarity of some of the materials may justify a brief discussion of the tentative formulas and of a proposed general principle upon which certain of them are based. Greater caution has been exercised in treating the more consequential problem of the biogenetic relationships between the different natural products, and the interpretations have not been extended beyond the limits of substantial evidence.

Some indication of the increasing activity in the phenanthrene field is afforded by the observation that about 45 per cent of the literature citations of the present volume are to papers published in the five-year period

1930-34, while the year 1935 alone accounts for no less than 23 per cent of the total. In venturing to present a review at a time when active interest is at a peak, it is felt that although the subject probably will expand rapidly in the near future the new observations may be of such a nature as to extend the present views without necessitating their drastic revision. In tune with the spirit of the investigations, every effort has been made to keep the book reasonably up to date at least at the time of publication, and through a series of fortunate circumstances it has been possible to review the literature received to February 1, 1936, with the exception of two or three papers which could be cited only in the notes.

It is a pleasure to acknowledge the coöperation and advice tendered by my colleagues and research assistants at Harvard. My wife and co-worker Mary Fieser has been of great help throughout the preparation and publication of the monograph, and she is responsible for the indexes. For reading parts of the manuscript dealing with their special fields and offering valuable criticism, I am greatly indebted to Dr. W. M. Allen, Dr. K. K. Chen, Dr. G. W. Corner, Dr. E. Fernholz, Dr. E. Mosettig, Dr. C. R. Noller, Dr. S. Palkin, Dr. M. J. Shear, and Dr. L. F. Small. In recognition of a valued source of counsel and inspiration in the course of this and other scientific enterprises, I may record that, only four days before his death, my esteemed friend Dr. Samuel C. Hooker had the spirit and kindness to verify the statements concerning the classical investigations of retene which he completed over fifty years ago.

L. F. F.

Cambridge, Massachusetts
February, 1936.

PREFACE TO THE SECOND EDITION

The fields of investigation falling within the scope of this book have been subject to such increased activity and interest during the past year that it has seemed desirable to keep pace in some measure with the rapid advances even before such a time as an extensive revision of the text may be called for. To this end, a survey of recent literature has been included in the second edition in the form of an appendix, and the indexes have been extended to accommodate this added material. The revising of the original text has been limited to the correction of typographical errors and to minor changes requiring no alteration in the pagination. The page references in the appended material consequently apply to the text of both editions and the separate publication of a supplement including the appendix and the revised indexes will prevent the devaluation of a first edition which is less than one year old.

Since the period for review in the appendix corresponds very nearly with the span of the year 1936, an effort has been made to include complete references to recent papers bearing publication dates up to January 1, 1937. Through the courtesy of the authors concerned, it has been possible to review a few additional papers which are in process of publication. The extraordinary activity in the phenanthrene field is well shown by the number of papers which have appeared in the brief period covered in the survey. The appendix includes references to over 300 papers published in 1936 as compared with about 200 citations to 1935 papers in the first edition. In presenting the new developments, it has seemed expedient to review as well some 50 earlier papers not previously included.

Although the year just passed has witnessed the isolation of a few additional natural products, including the highly important vitamin D, the period has been characterized less by dramatic disclosures comparable with those of the preceding years than by the systematic development of the fundamental chemistry of the polynuclear compounds, by the further elaboration of methods of interconversion and of synthesis, and by the general filling in of details to a pattern already clearly outlined. The future offers promise of much further profitable research along these lines, and active experimentation can be expected to continue. The delineation of the complete stereochemistry of the steroids is a particularly pressing problem, and further significant advances can be looked for in the near future. The time may not be far distant when the whole

matter of stereochemical nomenclature can be placed upon a thoroughly secure and rational basis, and until this point is reached it seems desirable to adhere to a temporary system which avoids the assumption of configurational relationships which still remain to be determined. A conservative proposal in this direction is given in the appendix.

The inclusion of new wax engravings in the appended material has not seemed justified. Revisions in structural formulas necessitated by the results of recent work are confined for the most part to minor points which can be appreciated without difficulty by reference to the earlier formulas. In the case of the triterpenoid saponins and the toad poisons, the knowledge of the structures is still very incomplete and tentative formulations suggested for the compounds have been subject to rapid change.

I am greatly indebted to a number of individuals for calling attention to errors in the original text, for offering helpful comments and criticisms, and for furnishing reprints of pertinent papers, and I will appreciate similar courtesies in the future. Mary Fieser has again taken over the work of preparing the indexes, and her constant assistance in dealing with questions of orthography, in proofreading, and in other technical matters connected with the publication of the book has been invaluable.

Cambridge, Massachusetts

L. F. F.

February 1, 1937

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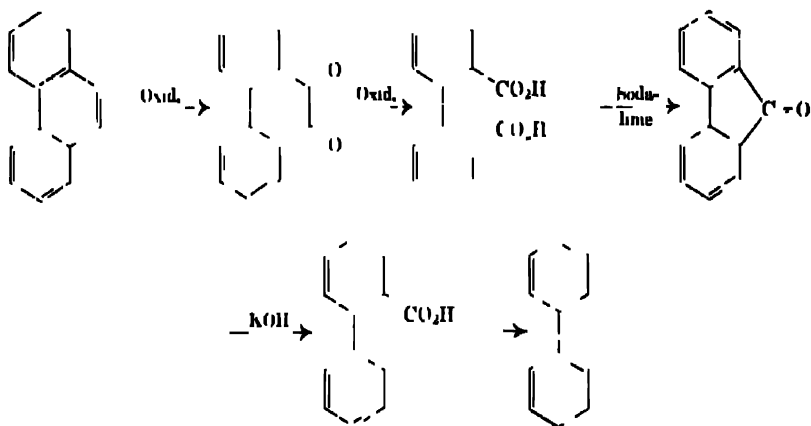
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Chapter I

The Chemistry of Phenanthrene and Some Instances of the Occurrence of Phenanthrene and Hydrophenanthrene Derivatives

The presence in coal tar of a hydrocarbon isomeric with anthracene was discovered independently in 1872 by Fittig and Ostermayer¹ and by Graebe,² the latter working with a product obtained by Glaser from technical anthracene. The formulas at present assigned to anthracene and phenanthrene appeared at the time of the discovery to be the only likely representations for either hydrocarbon in terms of the Kekulé theory, and Graebe and Liebermann³ were inclined to ascribe to anthracene the formula now assigned to the isomer. That the formulas should be interchanged was clearly shown by Fittig and Ostermayer in their first investigation of the new hydrocarbon, for they succeeded in establishing the structure of phenanthrene by the following degradation to diphenyl:



The degradation products other than diphenyl were unknown, but all of the transformations were correctly interpreted. The proof was completed by Schultz' synthesis⁴ of diphenic acid by a method which estab-

¹ Fittig and Ostermayer, *Ber.*, 5, 933 (1872), *Ann.*, 166, 361 (1874).

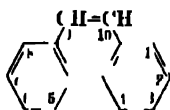
² Graebe, *Ber.*, 5, 461, 968 (1872), *Ann.*, 167, 131 (1873).

³ Graebe and Liebermann, *Ann. Spl.*, 7, 715 (1870).

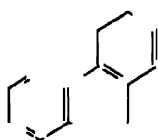
⁴ G. Schultz, *Ber.*, 11, 215 (1878), 12, 235 (1879).

lished its structure. In order to indicate the relationship to both diphenyl and anthracene, Fittig named the hydrocarbon phenanthrene.

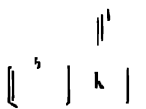
Phenanthrene may be regarded as a derivative of diphenyl with an ethylene bridge inserted between the *o*, *o'*-positions. That a double bond



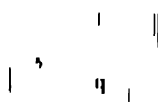
is located at the 9,10-position, and that it is more highly unsaturated than the double linkages of an isolated benzene ring, is clearly shown by the ability of phenanthrene to form a 9,10-addition product with bromine, as noted by the discoverers of the hydrocarbon. Only three of the four possible Kekulé bond structures for phenanthrene meet this requirement, namely those indicated in formulas I-III. On the basis of



(I)

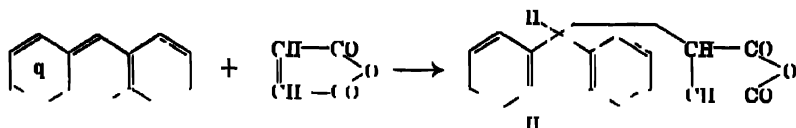


(II)



(III)

the rule that each ring of a polynuclear aromatic compound strives to assume as nearly as possible the condition of an isolated benzene ring,⁵ structure I represents a more stable arrangement of the bonds than II or III, for each of the three rings has a benzenoid structure. In the case of II and III both terminal rings are benzenoid, but the central nucleus of II has the structure of a tetraketone (k), while that of III is *o*-quinonoid (q), or dihydride in character. Theoretically phenanthrene should have the structure of I, and there is evidence that this is the case and that the bonds occupy fixed positions. The failure of the hydrocarbon to enter into the Diels-Alder reaction with maleic anhydride⁶ indicates the absence of an active diene grouping similar to that present in the central ring of anthracene:⁷



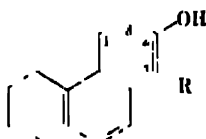
⁵ Fittig, Walter and Nehlring, *Ann.*, **516**, 248 (1935).

⁶ Clar, *Ber.*, **65**, 440 (1932).

⁷ Diels and Alder, *Ann.*, **486**, 101 (1931); Clar, *Ber.*, **64**, 1676, 2194 (1931).

Formulas II and III would allow of such addition since each contains a diene system, terminating at C_4-C_5 and at C_8-C_9 , respectively. The argument is somewhat weakened by the fact that the Diels-Alder reaction is reversible, but the observation shows at least that II and III are not the predominant tautomeric forms.

The behavior of the phenanthrols provides a still more definite indication of the bond structure.⁸ 2-Phenanthrol ($R = H$) couples with diazotized amines at position C_1 , a reaction which clearly involves the enolic double bond $-C(OH)=CH-$. An alkyl group at C_1 ($R = \text{alkyl}$) completely blocks the coupling reaction, from which it may be inferred that the bonds cannot migrate in such a way as to provide



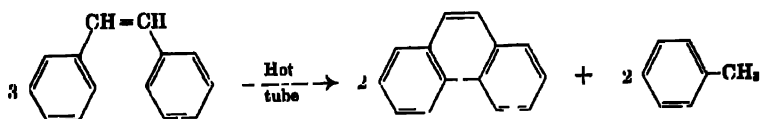
an enolic grouping at the free ortho position C_1 . The behavior of 3-phenanthrol similarly indicates the presence of a double bond at C_1-C_4 and the absence of such a linkage at C_2-C_1 . The failure of the allyl ether of 2-phenanthrol to rearrange when the normal ortho position (C_1) is blocked provides additional evidence. Phenanthrene appears to have a structure similar to that of naphthalene and with equally immobile double bonds.

Phenanthrene is more reactive than naphthalene and less reactive than anthracene. Of the two tricyclic hydrocarbons the linear isomer is the more easily oxidized or reduced. Polynuclear hydrocarbons containing both systems are oxidized chiefly to anthraquinones (para) rather than to phenanthrenequinones (ortho). According to the observations of Fries, Walter and Schilling,⁵ the heat of combustion of phenanthrene is 7.0 kilogram calories less than that of anthracene, indicating a lower energy content, and the relationship is well interpreted by the bond structures indicated above. That phenanthrene surpasses naphthalene in reactivity is indicated particularly clearly in the smoother conversion of the former hydrocarbon into its quinone by oxidation and by its ability to form an addition compound with bromine. The seat of special reactivity is the double bond between carbon atoms 9 and 10, each of which may be considered as an α -position of a naphthalene nucleus. The degree of unsaturation is distinctly greater than in the case of a double bond of benzene and in some respects the group approaches the character of an aliphatic linkage. Fries⁵ has suggested that the two other double

⁸ Fries and M. N. Young, *J. Am. Chem. Soc.*, **53**, 4120 (1931).

bonds of the central nucleus are so subject to the valence claim of the terminal rings of which they are also members that the conjugation with the C_9-C_{10} bond is disturbed and this linkage is left in a comparatively isolated condition. The above formula shows this condition of the bonds.

The Preparation of Pure Phenanthrene. Phenanthrene is a much less abundant constituent of coal tar than anthracene, and no convenient method has been discovered for the synthetic production of the hydrocarbon in quantity. Pyrolytic syntheses such as that of Graebe² are entirely unsatisfactory with respect to the yield and the purity of the



product. The phenanthrene present in coal tar is found in the anthracene-oil fraction, for phenanthrene and anthracene boil at nearly the same temperature. Phenanthrene is more soluble in organic solvents and technical material containing over 50% of phenanthrene is obtained by crystallization. By further crystallization of technical phenanthrene it is possible to remove the bulk of the anthracene and other impurities and to obtain colorless preparations melting at temperatures from about 102° to 115° . Pure phenanthrene, however, melts at approximately 101° ,⁹ and the material purified only by physical methods contains appreciable quantities of anthracene, carbazole, and fluorene. Phenanthrene forms with these substances mixed crystals having melting points higher than that of the pure hydrocarbon.¹⁰ Anthracene (m.p. 217°) present in amounts higher than about 2% raises the melting point of the isomeric hydrocarbon.

Since a separation cannot be achieved by the use of solvents alone,¹¹ it is necessary to resort to chemical treatment. E. Schmidt¹² introduced a convenient method which depends upon the greater ease of oxidation of anthracene as compared with phenanthrene. The solubility of anthraquinone in a solvent like alcohol is such that it is easily separated from phenanthrene. The crude phenanthrene is heated in alcoholic solution with a quantity of nitric acid sufficient to oxidize the anthracene present. This is converted into anthraquinone and dinitroanthraquinone, which precipitate, and the phenanthrene is obtained from the filtrate. The

⁹ Synthetic material

¹⁰ Pascal, *Bull. soc. chim.*, [4] 29, 544 (1921).

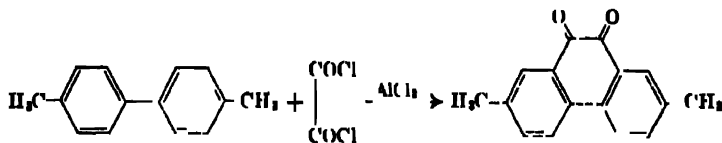
¹¹ J. M. Clark, *Ind. Eng. Chem.*, 11, 204 (1919).

¹² E. Schmidt, *Ber.*, 7, 205 (1874).

hydrocarbon is then distilled and further crystallized. The method has been improved in some details by others,¹³ and in order to obviate the danger of explosions during the distillation the partial oxidation has been carried out with the use of chromic acid in glacial acetic acid solution.¹⁴ The oxidation method cannot be relied upon for the removal of the last traces of anthracene, but this can be accomplished by boiling a solution of the purified hydrocarbon in nitrobenzene with maleic anhydride.¹⁵ Material required for hydrogenation experiments is best heated with sodium at 200° and distilled¹⁶ (b.p. 340°). The sodium removes a sulfur compound, probably diphenylene sulfide.

On a small scale the purification of phenanthrene in the form of its molecular compound with picric acid is often useful. The picrate crystallizes as yellow needles (m.p. 145°) from a solution of the components in alcohol, and the hydrocarbon can be recovered by distribution between aqueous ammonia or soda solution and ether. Molecular compounds useful for purposes of identification are formed also with styphnic acid (2,4,6-trinitroresorcinol), trinitrobenzene, trinitrotoluene, and 2,7-dinitroanthraquinone. Pure phenanthrene forms triclinic plates. It shows a blue fluorescence in solution, although this is less pronounced than in the case of anthracene.

Synthetic Methods. The only generally useful methods for the synthesis of derivatives of either phenanthrene or phenanthrenequinone are those which were developed for the purpose of identifying degradation products of naturally occurring substances or with the object of simulating these substances. These methods will be discussed in connection with the problems from which they originated. Among isolated instances of other methods of constructing the angular three-ring system, mention may be made of three direct syntheses of phenanthrenequinones. In two instances Liebermann¹⁷ was able to obtain alkylated phenanthrenequinones by the action of oxalyl chloride on alkylated diphenyls in the presence of aluminum chloride, for example:



¹³ Sandqvist, "Studien über die Phenanthren-sulfosäuren" Inaugural Dissertation, Uppsala, p. 18 (1912). F. L. Cohen and Cormier, *J. Am. Chem. Soc.*, **52**, 4368 (1930).

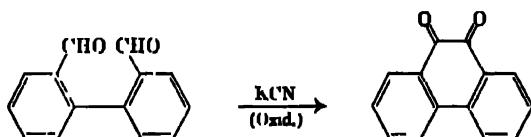
¹⁴ Mortimer and Murphy, *Ind. Eng. Chem.*, **15**, 1140 (1923); Buchmann, *J. Am. Chem. Soc.*, **57**, 555 (1935).

¹⁵ Clar, *Ber.*, **65**, 852 (1932).

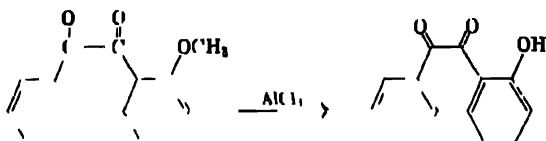
¹⁶ Schroeter, *ibid.*, **57**, 2025 (1924); Schroeter, H. Müller and Huang, *ibid.*, **62**, 645 (1929).

¹⁷ Liebermann, *ibid.*, **44**, 1453 (1911), **45**, 1186 (1912); Liebermann and Kardon, *ibid.*, **46**, 198 (1913).

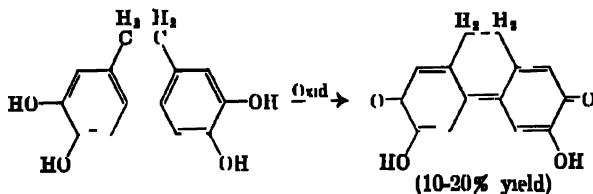
With diphenyl itself, however, and with all of the other derivatives tried, the only products isolated were carboxylic acids. Mayer¹⁸ succeeded in obtaining phenanthrenequinone in unspecified yield as a product of the reaction of diphenyl-*o,o'*-dialdehyde with alcoholic-aqueous potassium cyanide:¹⁹



Probably the reaction involves a benzoin condensation and a tautomeric shift to a dienol (hydroquinone), followed by air oxidation. Mayer applied the reaction in two other cases, but the aldehydes are not sufficiently available to make the method capable of any but very limited application. Another synthesis was achieved by Scholl and Schwarzer,²⁰ who obtained phenanthrenequinone in 25% yield by heating benzil with aluminum chloride at 120°. The most serious limitation of the method appears to be in the matter of yield. Brass, Willig and Hanssen²¹ obtained 1-hydroxyphenanthrenequinone by this method, but in only 1% yield:



An interesting instance of the formation of the reduced phenanthrene ring system by the establishment of the "diphenyl" linkage has been reported by Erdtmann:²²



Reaction with Bromine. The fact that phenanthrene combines with bromine to form an addition product which is sufficiently stable to be

¹⁸ T. Mayer, *Ber.*, **45**, 1105 (1912), **47**, 406 (1914)

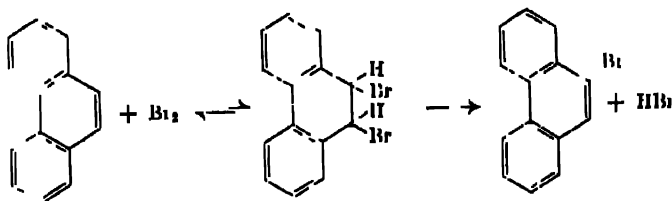
¹⁹ Kenner and Turner, *J. Chem. Soc.*, **99**, 2101 (1911), previously had reported negative results

²⁰ Scholl and Schwarzer, *Ber.*, **55**, 324 (1922)

²¹ Brass, Willig and Hanssen, *ibid.*, **65**, 2613 (1930)

²² Erdtmann, *Ann.*, **505**, 195 (1933)

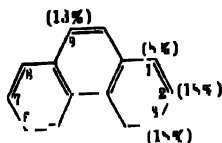
isolated and purified was noted in the first investigations of the hydrocarbon. On being heated, the addition product loses hydrogen bromide with the formation of 9-bromophenanthrene which, thanks to this reaction, is one of the most readily available derivatives of the hydrocarbon.



In pure solvents and in the absence of catalysts the addition reaction is reversible and proceeds with measurable velocity.²³ At temperatures from 0° to 40° the reaction (of equimolecular quantities of reagents) proceeds to a measurable point of equilibrium. The formation of a substitution product through an intermediate addition supports the view that this represents the mechanism of the bromination of aromatic hydrocarbons in general. That the addition product can be isolated in the case of phenanthrene (and anthracene) is due to the unusual reactivity of the hydrocarbon, the reaction proceeding easily under conditions so mild that the addition product is not decomposed. The metal halides employed as catalysts in ordinary brominations probably promote the elimination of hydrogen bromide from the intermediate and only accelerate the addition reaction by catalyzing the irreversible process by which the addition product is removed from the equilibrium.

Substitution Reactions. Contrary to what might be expected, the central nucleus of phenanthrene is not the exclusive point of attack in substitution reactions. In the nitration and sulfonation of the hydrocarbon and in the Friedel and Crafts reaction mixtures of isomeric mono- and di-derivatives usually are produced. Substitution in one terminal nucleus apparently does not greatly influence the ease of reaction in the second terminal ring. Since no less than five monosubstitution products and twenty-six disubstitution products are theoretically possible, it is clear that very complicated mixtures often result. The separation of these mixtures presents many difficulties, for many phenanthrene derivatives have a pronounced tendency to remain in supersaturated solution and to form mixed crystals. The phenomenon of polymorphism also is common. For these reasons the isolation of a pure product in 20-25% yield often is regarded as a highly satisfactory result, and relatively little is known concerning the complete course of the substitutions.

The nitration of phenanthrene in glacial acetic acid solution with diacetyl ortho nitric acid yields 9-nitrophenanthrene as the chief product, together with smaller amounts of the 2- and 4-derivatives and a very small amount of the 3-isomer.²⁴ The sulfonation of the hydrocarbon has been the subject of careful studies by investigators in four countries: A. Werner and his students, Sandqvist, Fieser, and Ioffé.²⁵ Werner isolated and characterized the 2-, 3-, and 9-acids and developed a method of separating the 2-acid as the barium salt. Fieser improved the methods of separation and identification (through the *p*-toluidine salts) and isolated in addition the 1-acid. The yields obtained on conducting the reaction at 60° are indicated in the formula. The 1-acid and the 9-acid do not appear among the products when the sulfonation is carried out for 3 hours at 120°, and the 2-acid and the 3-acid can be obtained fairly easily in 25-27% yields. The metal salts of the 2-acid are less soluble than those of the isomer and the organic salts melt at higher temperatures.



In analogy with naphthalene it would be expected that the 2- and 3-positions would be favored at an elevated temperature, for they correspond to β -positions, while positions 1, 4, and 9 are all α to an adjacent ring. It is well known that the α -acid of naphthalene, produced at a low temperature, rearranges to the β -acid at a higher temperature and, according to Ioffé, this is also true of phenanthrene. He concluded from time-yield curves that the 9-acid is formed first, even at a high temperature, and rearranges to the 3-acid. There were some indications that the 3-acid slowly rearranges to the 2-acid.²⁶

Sandqvist found that disulfonic acids accompany the monosulfonic acids even when phenanthrene is sulfonated incompletely at room temperature. When either the 2- or 3-sulfonic acid derivative is submitted to sulfonation,²⁷ the second substituent enters the unsubstituted, terminal nucleus chiefly at C₆ and C₇, and to a lesser extent at C₉.

When submitted to the Friedel and Crafts reaction with acyl halides under ordinary conditions, using carbon bisulfide as the solvent, phenan-

²⁴ Julius Schmidt and Heule, *Ber.*, **44**, 1496 (1911)

²⁵ Werner, *Frey J. Kunz, M. Kunz, Lowenstein, Rikner and Wark*, *Ann.* **321**, 214 (1902); Sandqvist *loc. cit.* *Ann.* **392**, 76 (1912); Fieser, *J. Am. Chem. Soc.*, **51**, 2460 (1929); Ioffé, *J. Gen. Chem. USSR*, **3**, 144 (1933)

²⁶ Fieser (*loc. cit.*) observed no rearrangements on heating the sodium salts of the 2-, 3-, or 9-acid with sulfuric acid, but this may not be a fair indication of the possible fate of the free acids

²⁷ Fieser, *J. Am. Chem. Soc.*, **51**, 2471 (1929)

threne is converted mainly into resinous products.²⁸ The results are no better when the reaction is moderated by operating at a low temperature or by employing the milder condensing agent stannic chloride, but Mosettig and van de Kamp²⁹ discovered that the reaction proceeds smoothly and simply in nitrobenzene solution. With acetyl chloride they obtained 3-acetylphenanthrene (m.p. 72°) in 64% yield and 2-acetylphenanthrene (m.p. 143°) in 15% yield. As with most other pairs of isomers, the 2-derivative is higher-melting and less soluble than the isomer and a separation is easily effected by crystallization. Benzoyl chloride and *o*-toluoyl chloride³⁰ react in the same solvent to give the 3-, 2-, and 1-derivatives in yields of 20%, 3%, and 6%, respectively. In the presence of aluminum chloride, succinic anhydride³¹ and methyl succinic anhydride³² also condense smoothly with phenanthrene in nitrobenzene solution and give chiefly the 3-substitution products (50-60% yield).

Possibly the superiority of nitrobenzene over other solvents is connected with the fact that it combines with aluminum chloride to form a rather stable molecular compound. The complex has sufficient catalytic activity to promote the desired reaction, but it apparently does not instigate side reactions to the same extent as the more potent, uncombined halide. Possibly the solvent also influences the direction of the substitution, but this point has not been fully investigated, other solvents giving inseparable or difficultly separable mixtures. Bachmann³³ used the Perrier modification of the Friedel and Crafts reaction to advantage in the separation of 1-benzoylphenanthrene from a mixture of isomers. Phenanthrene was added to a carbon bisulfide solution of the Perrier compound prepared from benzoyl chloride and aluminum chloride. A part of the reaction product separated as an insoluble complex and this on decomposition yielded exclusively the 1-benzoyl derivative in 8% yield. The separation is so simple that this ketone can be regarded as a readily available derivative in spite of the low yield.

Recently Burger and Mosettig³⁴ have found that 9,10-dihydrophenanthrene reacts far more smoothly than phenanthrene, and they obtained 2-acetyl-9,10-dihydrophenanthrene in 90% yield. This substance should

²⁸ The statements of Willgerodt and Albert, *J. prakt. Chem.*, **84**, 383 (1911), have not been confirmed. It has been shown by Mosettig and van de Kamp and by Bachmann (see below) that their "9-acetyl" and "9-benzoyl" derivatives do not have these structures.

²⁹ Mosettig and van de Kamp, *J. Am. Chem. Soc.*, **52**, 3701 (1930).

³⁰ Bachmann, *ibid.*, **57**, 555 (1935) Bachmann and Pincus, *ibid.*, **57**, 1130 (1935).

³¹ R. D. Haworth and Mavin, *J. Chem. Soc.*, 1012 (1933).

³² Cook and Haskwood, *ibid.*, 425 (1934).

³³ Bachmann, *J. Am. Chem. Soc.*, **57**, 553 (1935).

³⁴ Burger and Mosettig, *ibid.*, **57**, 2731 (1935).

furnish a convenient starting point for the preparation of many other derivatives.

In general the results with phenanthrene itself indicate that the 3-position is particularly favored in the Friedel and Crafts reaction. Substitution in the reactive central ring has not been observed and there is no evidence that acyl groups enter the 9-position and subsequently rearrange. Indeed Mosettig and van de Kamp³⁵ prepared 9-acetylphenanthrene (by the Claisen condensation of the 9-carbomethoxy derivative with ethyl acetate followed by hydrolysis of the keto ester) and found that the acetyl group does not migrate under conditions more drastic than in the Friedel and Crafts reaction.

The Preparation of Other Derivatives. Only a limited number of simple substitution products of phenanthrene are readily available as starting materials for reactions and syntheses. Among the ketones, 2-acetyl-, 3-acetyl-, and 1-benzoylphenanthrene fall into this category for they are available by direct substitution. 9-Benzoylphenanthrene is conveniently prepared by the condensation of 9-phenanthryl magnesium bromide with benzonitrile, followed by hydrolysis of the ketimine.³⁶ The 9-acetyl compound is prepared from the acid ester as described above. The 2-, 3-, and 9- ω -bromoacetyl derivatives of phenanthrene are easily obtained by bromination of the methyl ketones⁴⁷

The 2- and 3-carboxylic acids are most conveniently prepared by the action of aqueous sodium hypochlorite solution in excess on the corresponding acetyl compounds, the yields being nearly quantitative.³⁸ The corresponding nitriles can be prepared either from the acids or from the 2- and 3-sulfonates. Phenanthrene-9-carboxylic acid can be prepared from the 9-bromo compound either (1) through the Grignard reagent, by carbonation (70% yield), or by condensation with ethyl chlorocarbonate and hydrolysis of the ester (90% yield);³⁸ or (2) by heating the bromo derivative with cuprous cyanide at 260° and hydrolyzing the nitrile (84% yield).³⁹ 1-Phenanthroic acid has been obtained only from the difficultly accessible 1-sulfonate⁴⁰ and (in poor yield) by the alkali fusion of 1-benzoylphenanthrene.³³

The 2-, 3-, and 9-aldehydes have been prepared from the corresponding acid chlorides, (a)⁴¹ by the method of Rosenmund, the acid chloride

³⁵ Mosettig and van de Kamp, *J. Am. Chem. Soc.*, **55**, 3442 (1933)

³⁶ Buchmann, *ibid.*, **56**, 1367 (1934).

³⁷ Mosettig and van de Kamp, *ibid.*, **55**, 3449 (1933)

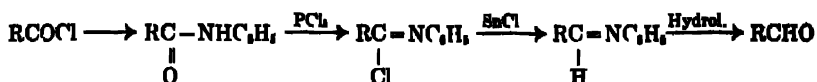
³⁸ Mosettig and van de Kamp, *ibid.*, **52**, 3704 (1930), **55**, 2995 (1933). According to later observations of these investigators (private communication) calcium hypochlorite ("ETH") is far superior to sodium hypochlorite for the oxidations.

³⁹ Mosettig and van de Kamp, *ibid.*, **54**, 3325 (1932)

⁴⁰ Hauser, *ibid.*, **54**, 4110 (1932)

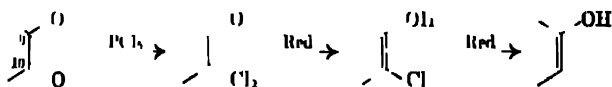
⁴¹ Mosettig and van de Kamp, *ibid.*, **55**, 2995 (1933)

being reduced catalytically in decalin solution (90% yields), and (b)^{38, 43} by the method of Sonn and Muller as follows (average yield, 58%):

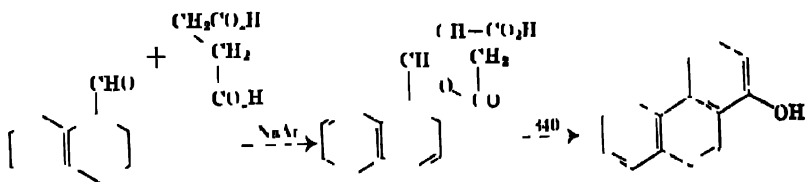


Bachmann⁴⁴ prepared 1-phenanthraldehyde by a process similar to (b) but starting with the anilide obtained by the Beckmann rearrangement of the oxime of 1-benzoylphenanthrene, the overall yield from the ketone being 60% of the theoretical amount.

All of the five possible phenanthrols are known but only 2- and 3-phenanthrol, which are easily prepared from the sulfonates,⁴⁴ are very readily available substances. Of various methods which have been suggested for the preparation of 9-phenanthrol, probably the most satisfactory consists in the reduction of 10,10-dichloro-9-phenanthrone,⁴⁵ best with stannous chloride in acetic acid saturated with hydrogen chloride.⁴⁶ Phenanthrenequinone is the starting material:



1-Phenanthrol has been obtained by the fusion of the rare 1-sulfonate⁴⁴ and, in poor yield, by the pyrolysis of α -naphthyl paraconic acid⁴⁷:



A more practical method has been described by Mosettig and Burger,⁴⁸ who obtained 1-phenanthrol in 50% yield by brominating 1-keto-1,2,3,4-tetrahydrophenanthrene and eliminating hydrogen bromide by the action of diethylaniline. 4-Phenanthrol has been obtained in small yield

³⁸ Shoppee, *J. Chem. Soc.*, 37, (1933)

⁴³ Bachmann, *J. Am. Chem. Soc.*, 57, 1341 (1935)

⁴⁴ For references and improved procedures, see Krieger, *ibid.*, 51, 2460 (1929)

⁴⁵ Julius Schmidt and Lumpp, *Ber.*, 41, 4215 (1908)

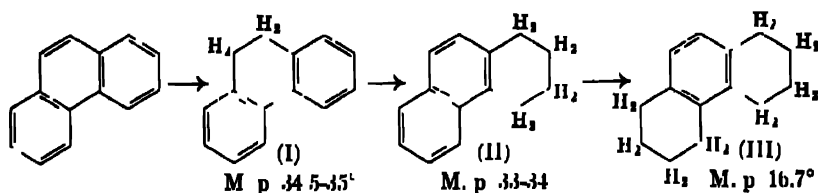
⁴⁶ Goldschmidt, Vogt and Brodig, *Ann.* 445, 135 (1925).

⁴⁷ Thorpe and Guthrie, *J. Chem. Soc.*, 2332 (1924)

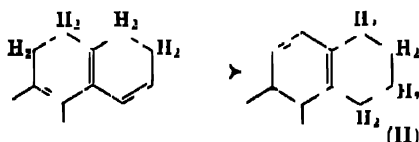
⁴⁸ Mosettig and Burger, *J. Am. Chem. Soc.*, 57, 2189 (1935)

from the condensation product of β -naphthaldehyde and succinic acid,⁴⁸ and the ethers of 1- and 4-phenanthrol have been produced synthetically by the Pschorr method (page 28).

Many of the early reports concerning various hydro derivatives of phenanthrene appear incomplete or inaccurate in the light of the careful studies of the high-pressure hydrogenation of phenanthrene carried out by Schroeter.⁵⁰ Three stages of hydrogenation were defined in this work and the structures of the highly purified hydrocarbons were all established by synthesis. Hydrogen atoms first enter the reactive central nucleus,



giving 9,10-dihydrophenanthrene (I). The next pair of hydrogen atoms probably saturates one of the double bonds of a terminal ring such as that at C₁-C₂, giving a tetrahydro compound which has two dihydro-benzenoid rings and which consequently is unstable. By a migration of one double linkage into the central nucleus, this isomerizes to the more stable structure II:



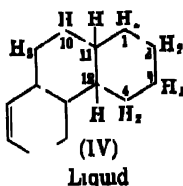
The symmetrical octahydride ("octanthrene") III is the final product and it can be prepared in 70-85% yield from phenanthrene,⁵¹ purification being accomplished through the sparingly soluble sodium sulfonate (Schroeter). 1,2,3,4-Tetrahydrophenanthrene (II) is the only hydro derivative of phenanthrene which forms a picrate, for it alone contains an intact naphthalene grouping. While the hydrocarbon can be isolated from the hydrogenation mixture in a pure form through the picrate, it is more conveniently prepared by synthesis (page 72). 9-10-Dihydrophenanthrene (I) was not obtained by Schroeter in quantity, for it was necessary to separate the substance from a mixture consisting largely of the other two hydrocarbons. After removing II as the picrate the mix-

⁴⁸ Behrend and Ludwig, *Ann.*, 379, 351 (1911)

⁴⁹ Schroeter, *Ber.*, 57, 3025 (1924); Schroeter, II. Möller and Huang, *ibid.*, 62, 645 (1929).

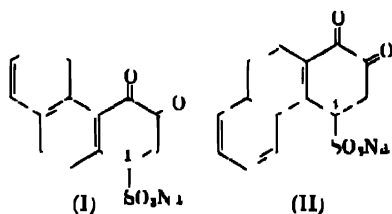
⁵¹ van de Kamp and Mowettig, *J. Am. Chem. Soc.*, 57, 1107 (1935)

ture is sulfonated. The dihydro compound forms a readily soluble disulfonate which can be obtained after removing the less soluble monosulfonate of 1,2,3,4,5,6,7,8-octahydrophenanthrene (III). More recently Burger and Mosettig⁸⁴ have found that pure 9,10-dihydrophenanthrene can be prepared in any desired amount by a process of selective hydrogenation, using copper-chromium-barium oxide catalyst.



The unsymmetrical isomer of III, 1,2,3,4,9,10,11,12-octahydrophenanthrene (IV), is readily available in quantity by synthesis⁸⁵ (page 76).

Further Observations Regarding Substitutions. A peculiarity noted in the reactions of phenanthrene is that substituting reagents almost invariably avoid the 4-position, possibly because of the spatial requirements of the molecule. That groups occupying the 4-position may be subject to steric hindrance is indicated by the striking difference in the reaction of the quinones I and II with aniline.⁸⁶ The normal



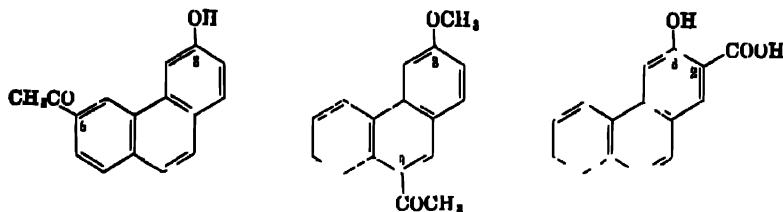
reaction for ortho quinone sulfonates of this type consists in the replacement of the sulfonate group by the anilino group, probably through the addition of aniline to the conjugated system of the quinone, followed by the elimination of sodium bisulfite. The quinone I reacts normally, giving the anilinoquinone, but in the case of II the 4-sulfonate group remains intact and the sodium salt is merely converted into the aniline salt. The remarkable stability of the sulfonate group in this position may be due to the influence of the neighboring benzene nucleus in preventing the formation of an intermediate addition product. Possibly

⁸⁴ Bardhan and Sen Gupta, *J. Chem. Soc.*, 2520 (1932); Bogdan, *Science*, 77, 269 (1933); Cook and Hewitt, *J. Am. Chem. Soc.* 1098 (1933); Bergs, *Ber.* 67, 278 (1934).

⁸⁵ Fieser, *J. Am. Chem. Soc.*, 51, 940 (1929).

the stereochemistry of diphenyl finds some counterpart in the properties of the tricyclic hydrocarbon which contains the diphenyl grouping.⁵⁴

Some rather unusual substitution reactions of the phenanthrols have been reported by Mosettig and Burger.⁵⁵ Treated with two molecules of acetyl chloride in nitrobenzene solution in the presence of aluminum chloride, 3-phenanthrol is converted into the 6-acetyl derivative, while the methyl ether under similar conditions yields the 9-derivative. The prod-



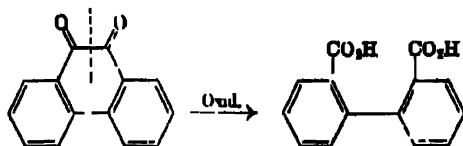
uct of the Kolbe reaction of sodium 3-phenanthrolate with carbon dioxide, according to these authors, very probably is 2-carboxy-3-phenanthrol. In all of these cases substitution would be expected to occur at the 4-position, as in the coupling reaction and in the Gattermann aldehyde synthesis. Mosettig and Burger found that the "normal" hydroxy acid, 4-carboxy-3-phenanthrol, is rather unstable, the carboxyl group being easily displaced on heating. 2-Phenanthrol is substituted at C₁ in the Friedel and Crafts reaction and in the Kolbe reaction, but its acetyl derivative reacts abnormally, giving either the 6- or 7-acetyl compound in the Friedel and Crafts reaction.

The Oxidation of Phenanthrene. Phenanthrenequinone can be prepared easily and in fair yield by the oxidation of the hydrocarbon in glacial acetic acid solution with chromic anhydride, or, more economically, in aqueous suspension using dichromate mixture.⁵⁶ The orange-colored ortho quinone is conveniently purified through the colorless, water-soluble addition product which it forms with sodium bisulfite, the quinone being precipitated on acidification of the aqueous solution (Graebe²). All traces of anthraquinone can be removed in this way. The chief difficulty in obtaining a high yield is that the quinone is easily oxidized further to diphenic acid by cleavage of the linkage between the two carbonyl groups. The reaction often is of value in determining the structure of substituted phenanthrenequinones, the oxidation being accomplished con-

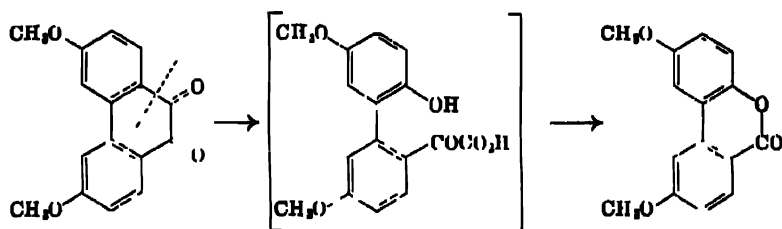
⁵⁴ It has been tentatively suggested [Fiener, *J. Am. Chem. Soc.*, **51**, 3101 (1929)] that the two benzenoid rings of 9,10-phenanthrenequinone may be distorted from the coplanar configuration, the twisting resulting in a strain within the molecule.

⁵⁵ Mosettig and Burger, *J. Am. Chem. Soc.*, **55**, 2041 (1933), Burger and Mosettig, *ibid.*, **56**, 1745 (1934).

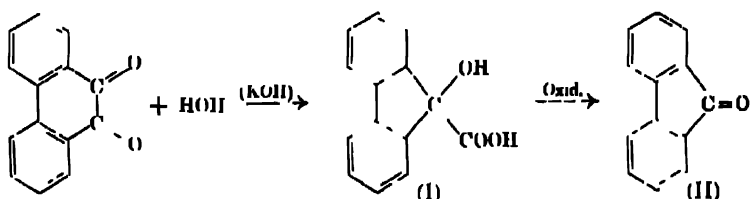
⁵⁶ Anschütz and G. Schultz, *Ann.*, **196**, 37 (1870), Oyster and Atkins, *J. Am. Chem. Soc.*, **43**, 208 (1921).



veniently by means of 30% hydrogen peroxide in glacial acetic acid solution.⁵⁷ In one instance²⁷ there has been observed a by-product which evidently results from the cleavage of the linkage between one ketone group and a benzene ring, followed by further oxidation and lactonization:

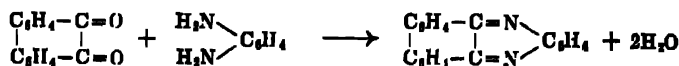


Like open chain α -diketones, phenanthrenequinone is sensitive to alkali and easily undergoes the benzilic acid rearrangement, giving hydroxyfluorene carboxylic acid I^{bb}:



Being an α -hydroxy acid, I is converted on oxidation into fluorenone (II), and consequently this ketone is the product obtained on oxidizing phenanthrenequinone with alkaline permanganate.⁵⁸

Phenanthrenequinone (m. p. 206°) has marked basic properties and forms oxonium salts with strong acids and with various metal halides. It is distinguished by various color tests, by the reduction to a colorless hydroquinone, and by its solubility in bisulfite solution. The quinoxaline derivative (phenanthraphenazine), prepared by condensation with *o*-phenylenediamine in glacial acetic acid solution, is useful for purposes of identification:



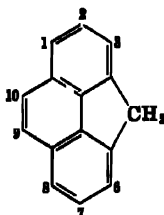
^a Hollman, *Rec. trav. chim.*, **23**, 169 (1904)

^b Friedländer, *Ber.*, **10**, 534 (1877); Julius Schmidt and Bauer, *ibid.*, **35**, 3757 (1905); H. Klinger, *Ann.*, **389**, 237 (1912).

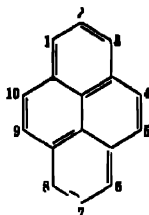
^c Anschütz and Japp, *Ber.*, **11**, 211 (1878)

OTHER COAL TAR HYDROCARBONS
RELATED TO PHENANTHRENE

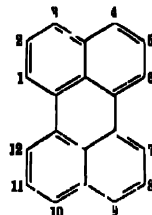
Among the hydrocarbons isolated from the distillates of coal tar or lignite tar which include as part of their structures the angular three-ring system of phenanthrene, the most interesting are those indicated in the accompanying formulas⁶⁰ (and 1,2-benzpyrene, page 83).⁶¹



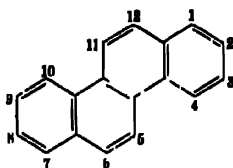
4, 5-Phenanthrylenemethane
(M. p. 116°)



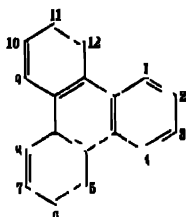
Pyrene
(M. p. 156°, corr)



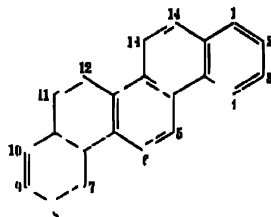
Perylene
(M. p. 265°)



Chrysene
(M. p. 255°, corr)



Triphenylene
(M. p. 190°)



Picene
(M. p. 365, corr)

The presence in coal tar of small quantities of 4,5-phenanthrylenemethane, perylene, and triphenylene has been established only recently. Prior to the isolation of perylene from coal tar,⁶² the synthetic hydrocarbon had been available by the cyclization of 1,1'-dinaphthyl derivatives with aluminum chloride.⁶³ Triphenylene, found present in crude chrysene to the extent of 1-3%,⁶⁴ was first obtained synthetically from cyclohexanone.⁶⁵ Under the influence of methyl alcoholic sulfuric acid three mole-

⁶⁰ Regarding the numbering of the hydrocarbons it may be observed that two systems are in current use in the case of pyrene and no less than four methods of numbering have been employed for chrysene. In these two cases, in the absence of strong precedent, the author has adopted the methods indicated for the following reasons: (1) they are consistent with the numbering generally accepted for phenanthrene, (2) they facilitate comparisons between the similarly located positions 1, 2, and 3 in phenanthrene, pyrene, and chrysene, and (3) by rational extension they adequately provide for all possible hydro derivatives of tar hydrocarbons. Pyrene and triphenylene have been numbered in a similar manner.

⁶¹ For a review of the literature of these compounds to 1927, see A. E. Everett, "The Higher Coal-Tar Hydrocarbons," Longmans, Green and Co., Ltd., London, 1927. Regarding perylene, see Houben "Das Anthracen und die Anthrachinone," pp. 577-583, Georg Thieme, Leipzig 1929.

⁶² Cook, Hewitt and Hieger, *J. Chem. Soc.*, 396 (1938).

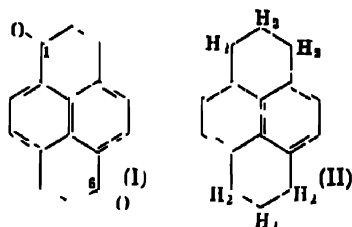
⁶³ Scholl and Weissenböck, *Ber.*, 43, 2202 (1910).

⁶⁴ Kaffar, *ibid.*, 68, 1812 (1935).

⁶⁵ Mannich, *ibid.*, 40, 153, 159 (1907).

rules of the ketone condense to give dodecahydrotriphenylene (compare: acetone→mesitylene), and the aromatic hydrocarbon can be obtained by dehydrogenation. The other hydrocarbons were all unknown prior to their isolation from tars. Kruber⁶⁶ isolated 4,5-phenanthrylene-methane by heating a purified neutral fraction of anthracene oil with sodium, followed by treatment with carbon dioxide. One of the (relatively acidic) hydrogen atoms of the methylene group is replaced by sodium and the substance is converted on carbonation into the sodium salt of the corresponding acid. The hydrocarbon is then obtained on heating the acid above the melting point. On oxidation the substance is attacked with about equal ease at the 9,10-position and at the methylene group.

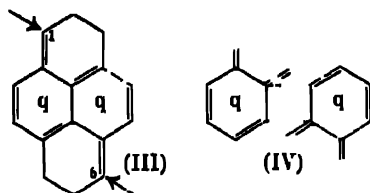
From such observations as have been made it appears that the structural resemblance of pyrene to phenanthrene is largely superficial, for the two hydrocarbons are quite different in their behavior. On being oxidized, pyrene does not yield an ortho quinone but is converted into a heteronu-



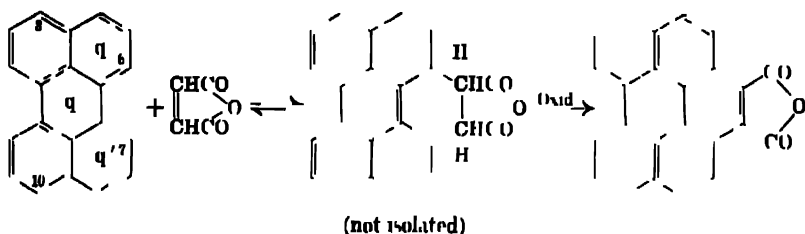
clear quinone which is known to be either the 1,6-(I) or the 1,8-derivative. Reduction with sodium and amyl alcohol gives chiefly symmetrical hexahydropyrene (II).⁶⁷ Whereas in the Friedel and Crafts reaction phenanthrene is attacked chiefly at the β -positions, C₂ and C₈, pyrene is substituted at position C₁, which is adjacent to a second aromatic ring. A second substituent enters a corresponding position in the other terminal ring, appearing largely at C₆ and to a lesser extent at C₉. Pyrene also is distinctly more reactive than phenanthrene. In explanation of these differences it may be noted that it is not possible for the hydrocarbon to assume a Kekulé bond structure such that each of the four rings has the stable benzenoid arrangement of linkages. The structure represented above and partially reproduced in III has two ortho quinonoid (q) nuclei (see IV), which are combined in such a way that the unsaturated systems of linkages terminate at C₁ and C₆. This may account for the predominant substitution at these points.

⁶⁶ Kruber, *Ber.*, 67, 1000 (1934)

⁶⁷ Cook and Hewett, *J. Chem. Soc.*, 398 (1933)



Perylene differs from all of the other hydrocarbons of the group in being colored (yellow), and it also departs widely from phenanthrene in properties. The hydrocarbon is converted by oxidizing agents chiefly into the 3,10-quinone, and, in general, substitutions occur at the 3,10- and, to a lesser extent, at the 3,9-positions. The bond structure indicated in the formula above is that suggested by Clar,⁸⁸ largely on the basis of his observation that perylene combines with malic anhydride when the two substances are heated in nitrobenzene solution. Apparently it is necessary to use this oxidizing solvent in order to displace the initial equilibrium:



The reaction indicates the presence of a diene system between C_6 and C_7 , and according to the formula these positions represent the ends of the unsaturated, quinonoid system of the central nucleus (q). The formula also accounts for the substitutions at C_1 and C_{10} , for these positions are at the ends of the quinonoid systems of rings q' and q'' .

Chrysene, triphenylene, and picene are more stable, unreactive substances and evidently contain only truly benzenoid rings. Chrysene and picene yield ortho quinones on oxidation, while no definite products have been obtained from triphenylene. It will be noted that the latter hydrocarbon lacks a reactive double bond comparable with that at C_6 - C_{10} in phenanthrene.

The Chrysogens of Coal Tar Distillates. Chrysene (Gr. *chrysos*, gold) was so named for the reason that the hydrocarbon prepared from coal tar (and by other pyrogenic methods) forms beautiful golden-yellow plates, the color being retained in only slightly diminished intensity after numerous crystallizations. Liebermann⁸⁹ discovered that the color is due

⁸⁸ Clar, *Ber.*, 65, 846 (1932).

⁸⁹ Liebermann, *Ann.*, 158, 200 (1871).

to an impurity which is attacked more easily than chrysene when the yellow material is subjected to partial oxidation or reduction, and he was able to prepare completely colorless chrysene (having a red-violet fluorescence). The colored material, or chrysogen, is not present in amount sufficient to influence greatly the physical or chemical properties (or the analysis), and the original name was retained as a matter of convenience. Colorless chrysene was encountered independently by E. Schmidt⁷⁰ in an interesting manner. Schmidt undertook the investigation of a supposed "nitro-anthracene" which had been obtained by the action of nitric acid on an alcoholic solution of technical anthracene. On careful examination the substance was found to be a molecular compound of chrysene and 2,7-dinitroanthraquinone, the latter compound evidently arising from the oxidation of anthracene, followed by nitration. On treatment with aqueous stannous chloride solution the nitro compound was reduced to a water-soluble amine salt, leaving chrysene as a gray precipitate. This apparently is the origin of the use of the sparingly soluble molecular compounds of 2,7-dinitroanthraquinone in the identification and purification of polynuclear aromatic hydrocarbons.

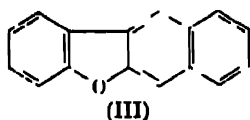
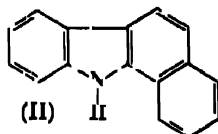
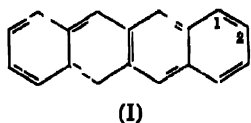
Clar⁷¹ has reported that the colored impurity can be removed by boiling a solution of chrysene in xylene with a small quantity of maleic anhydride. A more convenient method consists in shaking a warm solution of the hydrocarbon in tetrachloroethane with successive small portions of concentrated sulfuric acid until the acid liquor no longer becomes colored.⁷² All of the methods of purification indicate that the chrysogen is a relatively reactive substance, and Clar's observation suggests that it is an anthracene derivative, but the identity of this and other chrysogens was only established with the use of the method of chromatographic adsorption analysis by Winterstein, Schön and Vetter.⁷³ A nearly saturated solution of a crude hydrocarbon is prepared in a suitable mixture of benzene and ligroin and this is passed through a tower packed with finely powdered, activated (heated) alumina. The different constituents are selectively adsorbed, and on examination under ultraviolet light various zones can be detected in different parts of the tower. Each zone is removed and subjected to elution with ether in order to extract the organic material. In this way Winterstein and his collaborators identified the colored constituent of crude chrysene as naphthacene (I), a particularly reactive, linear benzologue of anthracene. They also found present the colorless, nitrogen-containing compound 1,2-benzocarbazole (II). Slightly

⁷⁰ E. Schmidt, *J. prakt. Chem.*, **9**, 241 (1874)

⁷¹ Clar and Lombardi, *Ber.*, **65**, 1411 (1932)

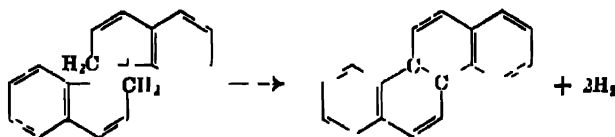
⁷² Observation of the author

⁷³ Winterstein, Schön and Vetter, *Z. physiol. Chem.*, **230**, 158 (1934)



yellow anthracene from coal tar was found to contain traces of naphthalene and carbazole. Pyrene from the same source contains a particularly persistent chrysogen and earlier investigators were of the opinion that the pure hydrocarbon is yellow. Purification with sulfuric acid is not applicable in this case because pyrene is too easily sulfonated. By the chromatographic method the compound was obtained in a colorless condition, the chrysogen was identified as 1,2-benzonaphthalene, and a colorless impurity was detected in small amounts and identified as brazan (III). According to von Braun and Irmisch,⁷⁴ technically purified chrysene from coal tar contains considerable amounts of sulfur compounds (1.4% S). These can be removed by treatment with sodium, but the process is wasteful.

Synthetic Preparation of Chrysene. There are many connecting ties in both the chemistry and the history of chrysene and phenanthrene, and it is appropriate to consider briefly the synthetic approaches to the four-ring hydrocarbon. Chrysene has been obtained in high-temperature reactions from a variety of different substances or mixtures of substances, the most striking and useful example being that discovered by Spilker.⁷⁵ This investigator obtained the hydrocarbon by passing indene vapor through a glowing iron tube and he reported that no less than 60% of the indene actually consumed in the process was converted into chrysene. The reaction possibly involves primarily the rupture of the allylic linkage of indene. So satisfactory is the yield that sulfur-free chrysene prepared

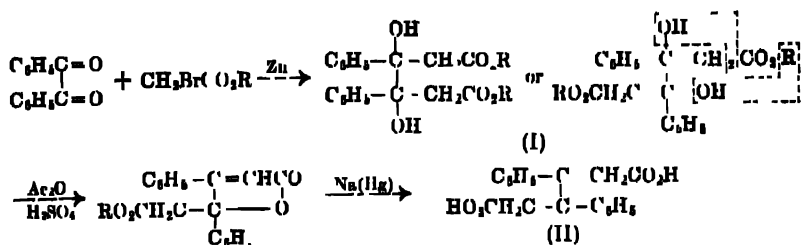


technically by Spilker's method vies in price with the material from coal tar.

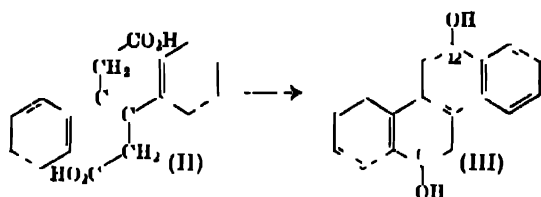
⁷⁴ von Braun and Irmisch, *Ber.*, **65**, 883 (1932)

⁷⁵ Spilker, *ibid.*, **26**, 1538 (1898).

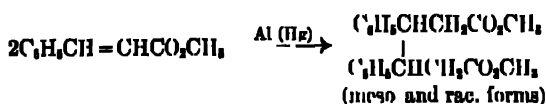
The first method of synthesizing chrysene derivatives of known structure was that developed by Beschke.⁷⁶ Benzil was condensed with α -bromoacetic ester in a Reformatsky reaction and the meso ester (I), after being separated from a small quantity of the racemic isomeride, was converted by dehydration and reduction into the dibasic trans acid II (β ,



γ -diphenyl- α,δ -dihydromuconic acid). With this material a double ring closure was accomplished by treatment with acetic anhydride-sulfuric



acid, giving, by the spontaneous aromatization of the original diketone, 6,12-dihydroxychrysene (III) as the acetyl derivative. A modification of the synthesis was introduced by von Braun and Irmisch,⁷⁷ who obtained two stereoisomeric esters suitable for the double cyclization by the dimolecular reduction of methyl cinnamate:



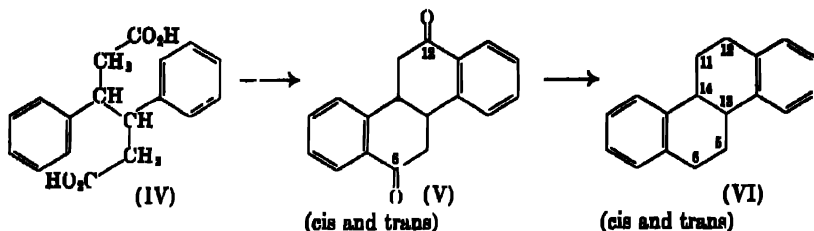
The corresponding acids (IV) on ring closure gave the cis and trans forms of diketohexahydrochrysene (V), and after reduction of the carbonyl groups the two 5,6,11,12,13,14-hexahydrochrysenes (VI) were converted into chrysene by dehydrogenation with selenium. The method has been improved and extended by R. Robinson⁷⁸ and by Vogel.⁷⁹

⁷⁶ Beschke, *Ann*, 384, 143 (1911)

⁷⁷ von Braun and Irmisch, *Ber*, 64, 2461 (1931)

⁷⁸ Ramage and R. Robinson, *J. Chem. Soc.*, 607 (1934); Lewis, Ramage and R. Robinson, *ibid*, 1412 (1935); R. Robinson and P. G. Young, *ibid*, 1414 (1935)

⁷⁹ Vogel, *Nature*, 131, 402 (1933)



Chrysene also has been prepared as follows by methods which are essentially phenanthrene syntheses and which will be described later: by the Pschorr method⁶⁰ (page 28), by the Perlman-Davidson-Bogert method⁶¹ (page 77), by the Bardhan-Sengupta (page 76) and Haworth (page 71) methods,⁶² and by a diene synthesis (page 110). Picene (*L. piceus*, pitch) has been synthesized by the second of these methods (Ruzicka and Hösli, *loc. cit.*). It is of interest that chrysene (b. p. 453°) distills with mercury⁶³ (b. p. 365.5°) at a temperature of 347°.

THELEPHORIC ACID, A FUNGUS PIGMENT

Zopf⁶⁴ in 1889 isolated from a fungus of the species *Thelephora* a pigment which forms beautiful crystals closely resembling potassium permanganate in color and which was called thelephoric acid. Many years later Kögl, in the course of his extensive studies of the fungus pigments, found an improved method of extraction with the use of pyridine and he and his collaborators⁶⁵ were able to collect 1.5 g. of thelephoric acid. With the use of micromethods this amount sufficed for a complete elucidation of the structure. Analyses and tests established the fact that the substance is a quinone having three hydroxyl and two carboxyl groups. The presence of the phenanthrene nucleus was determined by zinc dust distillation, followed by oxidation of the resulting hydrocarbon (II), the product being identified as phenanthrene-2-carboxylic acid (III). The character of the side chain was revealed when the hexahydro derivative (IV) was submitted to oxidation, one product being adipic acid (VI). A second acid, which was also obtained by the oxidation of thelephoric acid with alkaline hydrogen peroxide, was identified as the hydroxytrimellitic acid, V. The isolation of this degradation product establishes the structure of one of the terminal rings. The location of the two hydroxyl groups

⁶⁰ Weitsenböck and Iarb, *Monatsh.*, **33**, 549 (1912).

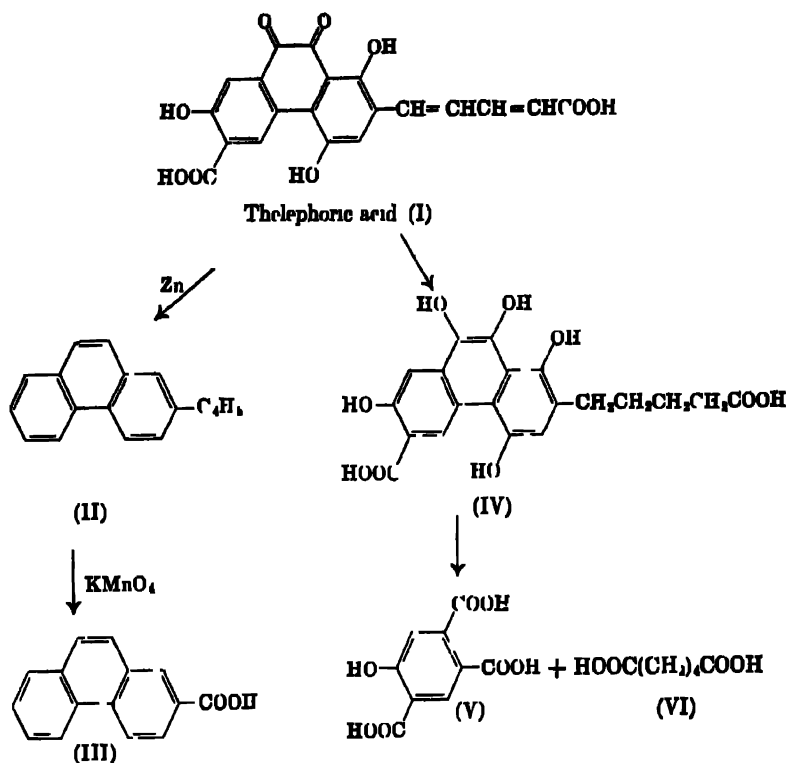
⁶¹ Cook and Hewett, *J. Chem. Soc.*, 1095 (1933), 365 (1934), Ruzicka and Hösli, *Helv. Chim. Acta*, **17**, 470 (1934).

⁶² R. T. Haworth and Maivin, *J. Chem. Soc.*, 1012 (1933).

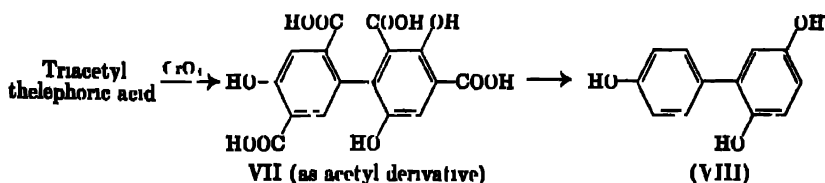
⁶³ Docker, *Ber.*, **67**, 1636 (1934).

⁶⁴ Zopf, *Bot. Zeitung*, **69** (1889).

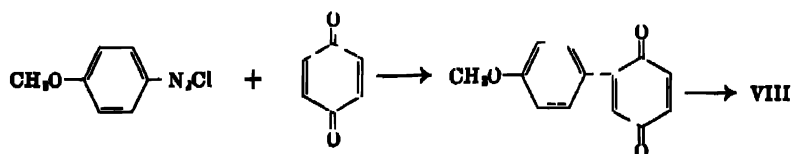
⁶⁵ Kögl, Eizleben and Jänecke, *Ann.*, **482**, 105 (1930).



in the ring carrying the side chain at position C₂ was determined in another degradation. On protecting the hydroxyl groups by acetylation, the quinonoid nucleus could be opened by oxidation, giving a diphenic acid derivative, VII. On decarboxylation this gave a trihydroxydiphenyl



(VIII), which was identified by synthesis, use being made of a special reaction of quinones with diazonium compounds:



On reduction of the substituted quinone, followed by demethylation, a product was obtained identical with VIII.

Xylindein ($C_{14}H_{16}O_{11}$), a green dye from decayed beechwood, also appears to contain the phenanthrene nucleus,⁵⁶ but the investigations of the structure of the substance are still far from complete.

PHENANTHRENE ALKALOIDS

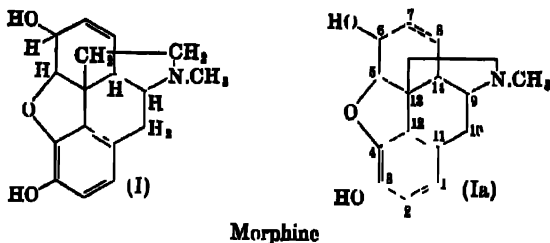
In the course of the investigations in the field of the alkaloids it has become apparent that a rather considerable number of these plant products contain as a characteristic part of the carbon framework a partially reduced phenanthrene nucleus. Without attempting a detailed review of the specialized and complicated chemistry of these substances, it will be of interest to consider briefly the characteristic features of their structures and the nature of the evidence upon which the structures are based. Of prime importance in the investigations was the degradation of the alkaloids to phenanthrene derivatives and the identification of these substances by synthesis, and it is this phase of the work which is of particular interest in connection with the other subjects included in the present volume.

The Morphine Group. Most prominent among the alkaloids of the phenanthrene group are the opium alkaloids morphine (Gr. *Morpheus*), codeine (Gr. *kodēia*, poppy head) and thebaine (from a kind of Egyptian opium produced at Thebes).⁵⁷ On account of the analgesic, hypnotic, and calmate properties of the alkaloids or their conversion products, these drugs have become indispensable in modern medical practice in spite of their dangerous habit-forming character, and the chemistry of the three related compounds has been the subject of active investigation since the discovery of morphine in 1803. Nearly twenty different morphine formulas have received consideration in the course of the work and, on account of the peculiarly intricate nature of the problem, the rigid proof of the structure of the alkaloid is still incomplete and the synthesis is a problem of the future. The formula of Gulland and Robinson,⁵⁸ however, although not proved beyond all question is generally accepted as representing the true structure of morphine. This formula, I, which is conveniently abbreviated as in Ia, represents the alkaloid as a hexa-

⁵⁶ Klotz and Ersleben, *Ann.*, **484**, 65 (1930).

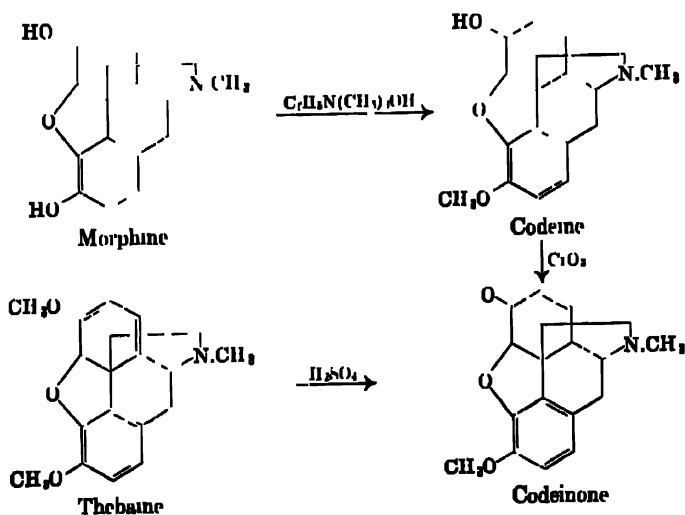
⁵⁷ For an excellent account of the chemistry of these substances, with complete references, see L. F. Small, "Chemistry of the Opium Alkaloids," U. S. Treasury Department, Supplement No. 103 to the Public Health Reports (1932).

⁵⁸ Gulland and R. Robinson, *Mem. Proc. Manchester Lit. Phil. Soc.*, **69**, 79 (1925).



hydrophenanthrene derivative having a phenolic hydroxyl group (C_3), a secondary alcoholic group (C_6), an oxide bridge between positions 4 and 5, and an ethanamine chain, $—CH_2CH_2N(CH_3)—$, inserted between positions 9 and 13 and constituting a part of a six-membered heterocyclic ring.

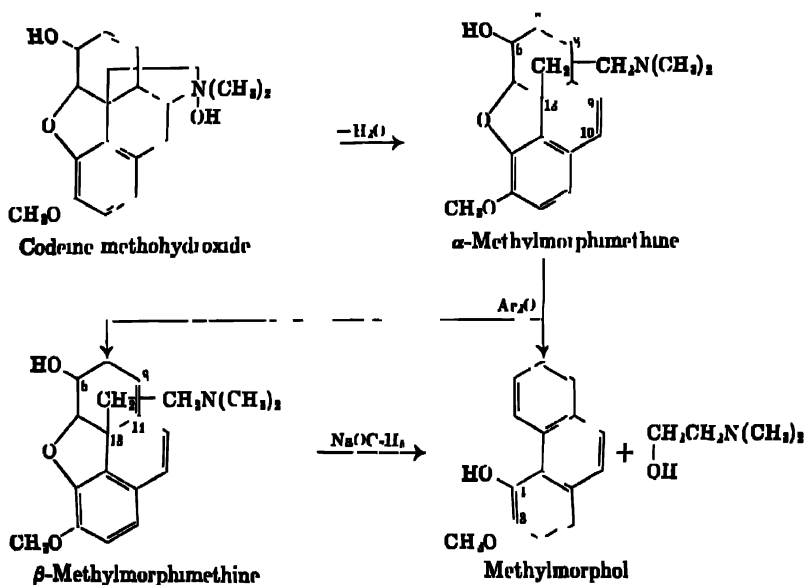
Codeine is the phenolic methyl ether of morphine and can be prepared from this substance by methylation, best with phenyltrimethylammonium hydroxide (which does not give quaternary N-alkyl compounds). Because of its relatively slight tendency to cause habituation, codeine is used extensively in medical practice, and since it is present in opium in smaller amounts (0.2-0.8%) than morphine (7-15%), the methylation process is one of considerable importance. Since it is a secondary alcohol, codeine



can be oxidized to the ketone codeinone, which forms a connecting link to thebaine. This alkaloid is the methyl ether of the enolic form of codeinone. On hydrolysis of the enol ether group with dilute acids, thebaine is transformed into codeinone.

The Morphol Cleavage. The formation of phenanthrene as a product of the zinc dust distillation of morphine was observed as early as

1881,⁸⁹ but by the use of gentler methods it was found possible to obtain substituted phenanthrenes which provided much more information regarding the structure of the alkaloid. When, for example, morphine methiodide is heated with acetic anhydride, the nitrogen is cleaved from the molecule and the chief product is the diacetyl derivative of 3,4-hydroxyphenanthrene, or morphol.⁹⁰ The changes occurring are best illustrated by an example of a stepwise degradation by the Hofmann exhaustive methylation method. The quaternary base obtained in solution by the action of alkali on codeine methiodide loses the elements of water when the solution is heated and yields α -methylmorphimethine. A double bond is introduced at C₉-C₁₀ on rupture of the nitrogen-containing ring.



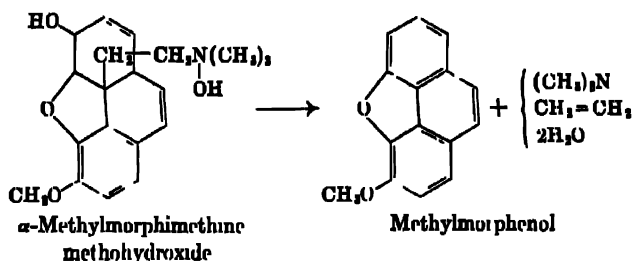
α -Methylmorphimethine on acetolysis yields the cleavage products methylmorphol (the 3-methyl ether of morphol) and ethanoldimethylamine, both substances being obtained as the acetyl derivatives. A part of the material escapes acetolysis by being converted into the more stable isomeride, β -methylmorphimethine, through the migration of the double bond from C₉-C₁₀ to C₈-C₉. The β -methine can be converted into methylmorphol by vigorous treatment with sodium ethylate. In partial explanation of the reactions it may be observed that in the case of each methine the central nucleus has a dihydrobenzenoid structure but that the side

⁸⁹ Vongerichten and Schrötter, *Ann*, 210, 396 (1881)

⁹⁰ O. Fischer and Vongerichten, *Ber*, 19, 792 (1886)

chain at C₁₁, offers an obstruction to the aromatization of this ring. The tendency to assume the completely aromatic condition evidently is so great that the obstructing group is eliminated. The reaction is complicated, however, for it involves also the elimination of the alcoholic group at C₆ and the opening of the oxidic bridge.

By a variation of the degradation the oxide ring is retained in the final product, as, for example, when α -methylmorphimethine is exhaustively methylated and the quaternary base submitted to thermal decomposition. A normal decomposition would involve only the loss of trimethylamine



and water, with the production of a vinyl derivative, but the reaction proceeds beyond this stage and gives a phenanthrene derivative from which the entire side chain has been eliminated.

Morphol and morphenol, in the form of their acetyl derivatives or ethers, were obtained as degradation products at an early stage in the investigations,^{90, 91} and the importance of determining the structures of these substances was recognized from the start. The chemistry of phenanthrene had been so little explored, however, that it was nearly twenty years before Vongerichten, from a series of simple but significant observations, succeeded in solving the problem. He had found that diacetyl morphol can be oxidized to a characteristic phenanthrenequinone (1886), and concluded that the two hydroxyl groups of morphol are not situated at C₆ or C₁₀. It was further observed⁹² that the quinone obtained on hydrolysis, morpholquinone (3,4-dihydroxy-9,10-phenanthrenequinone), has an affinity for mordanted fabrics which characterizes the substance as an ortho dihydroxyquinone similar to alizarin. A relationship between morphol and morphenol was established by the conversion of the latter substance into the former by reduction with sodium and alcohol.⁹³ At a later date it was shown that morphenol can be converted on hydrolytic cleavage with alkali into a trihydroxyphenanthrene.⁹⁴ Finally Vonge-

⁹⁰ Vongerichten and Schrotter, *Ber.*, 15, 1484 (1882).

⁹¹ *Idem*, *Ibid.*, 31, 2924 (1898).

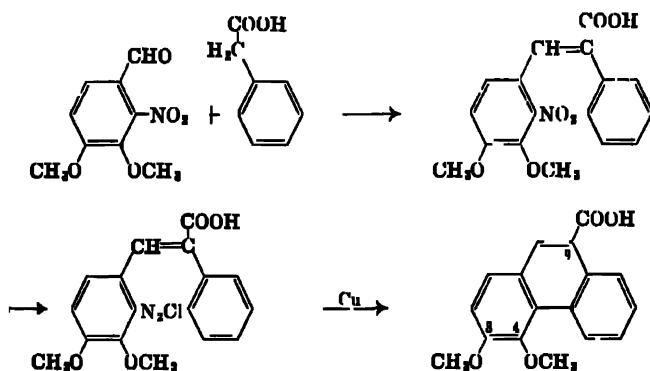
⁹² *Idem*, *Ibid.*, 31, 3198 (1898).

⁹³ *Idem*, *Ibid.*, 33, 1924 (1900).



richten⁹⁵ found that acetyl morphenol can be oxidized to a phenanthrene-quinone, showing that the oxide bridge is not linked to the central nucleus. Since a bridge between positions C_4 and C_5 is the only other reasonable possibility, and in view of the evidence that the two hydroxyl groups of morphenol occupy ortho positions, Vongerichten was able to assign to the two compounds the correct structures.

The Pschorr Synthesis. Recognizing the importance of providing a rigid proof of structure in these and other cases, Pschorr had undertaken an attack of the problem by the synthetic route, and in 1896 he developed a general method for the synthesis of phenanthrene derivatives⁹⁶ which was to have wide application in the subsequent investigations of phenanthrene alkaloids. Phenanthrene and some of its simple derivatives were prepared by the new method⁹⁷ and, following the appearance of Vongerichten's paper of 1900, Pschorr confirmed the structure assigned to morphenol by the synthesis of dimethylmorphol.⁹⁸ As applied in this case, the first step in the Pschorr synthesis consists in the condensation of vic-*o*-nitrovanillin methyl ether with sodium phenylacetate. The α -phenyl-*o*-nitrocinnamic acid is converted through the amine to the diazonium



salt, and under the catalytic influence of copper powder this loses nitrogen and hydrogen chloride and yields a phenanthrene-9-carboxylic acid.

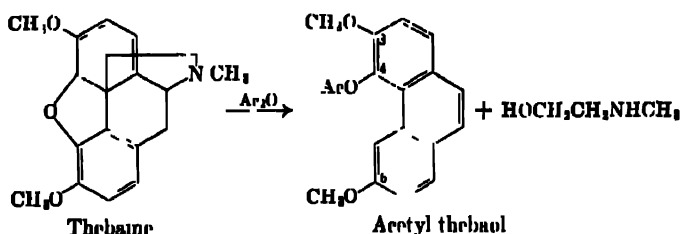
⁹⁵ Vongerichten, *Ber.*, **33**, 352 (1900)

⁹⁶ Pschorr, *ibid.*, **29**, 496 (1896)

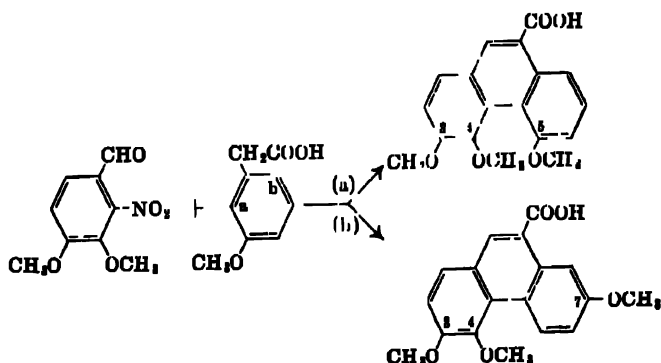
⁹⁷ Pschorr, Wolfes and Burkow, *ibid.*, **33**, 182 (1900), Pschorr, *ibid.*, **33**, 176 (1900)

⁹⁸ Pschorr and Sumuleanu, *ibid.*, **33**, 1810 (1900)

The product of decarboxylation in the above case proved to be identical with the dimethyl ether of morphol.⁸⁶ The method was found to be generally useful for the synthesis of phenanthrene derivatives of known structure,⁸⁹ and it was possible to establish the structures of the methylmorphol obtained from codcine, and of thebaol, a degradation product obtained as the acetyl derivative by the acetolysis of thebaine.



Substitution products of both *o*-nitrobenzaldehyde and phenylacetic acid can be employed in the Pschorr synthesis for the production of a wide variety of polysubstituted phenanthrenes, but a difficulty was encountered in the attempt to synthesize 3,4,5-trimethoxyphenanthrene for comparison with the trimethyl ether of the substance resulting from the hydrolysis of the oxide ring of morphenol.¹ Starting with vic-*o*-nitrovanillin dimethyl ether and *m*-methoxyphenylacetic acid, the phenanthrene ring closure of the diazonium salt theoretically can take place at either of the



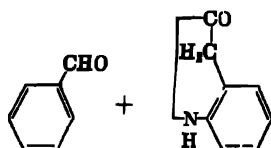
ortho positions *a* or *b* in the original ring of the methoxyphenylacetic acid. Pschorr found that a mixture of the 3,4,5- and the 3,4,7-trimethoxyphenanthrene-9-carboxylic acids indeed is produced, but he succeeded in overcoming the difficulty by blocking the ortho position *b* with a bromine

⁸⁶ Pschorr and co-workers, *Ber.*, **33**, 1926, 1927 (1907); **34**, 3798 (1901); **35**, 1400, 1412 (1902); **39**, 8106 (1906).

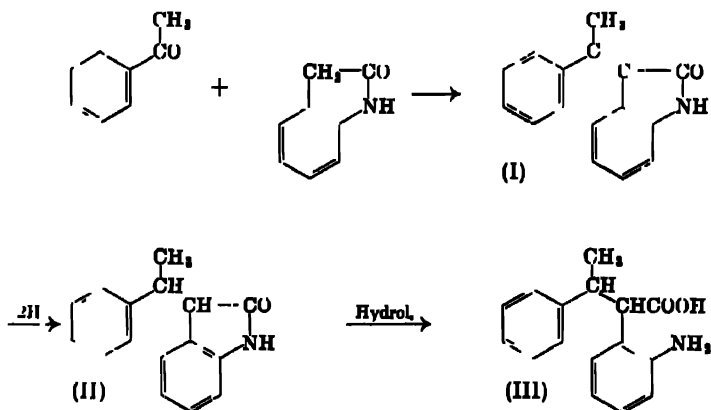
¹ Pschorr, *Ann.*, **391**, 40 (1912).

atom which subsequently was removed from the phenanthrene derivative by reduction. Mayer investigated other cases in which a ring closure in two directions is possible and likewise obtained mixtures,³ and he encountered difficulties in effecting the decarboxylation of certain alkylated 9-phenanthroic acids.³

An interesting modification of the Pschorr synthesis was introduced by Windaus,⁴ who was interested in preparing 9-methylphenanthrene⁵ for comparison with a degradation product obtained from the alkaloid colechicine (page 38). In place of phenylacetic acid Windaus used as the component with the active methylene group a substance oxindole, which also contains, in the position ortho to the side chain, a latent amino group which later is available for the ring closure:



One advantage of the method is that ketones can be employed in the condensation as well as aldehydes, making available 9-alkylphenanthrenes. The desired 9-methyl derivative was obtained from the starting materials acetophenone and oxindole. The condensation product (I) was hydroge-



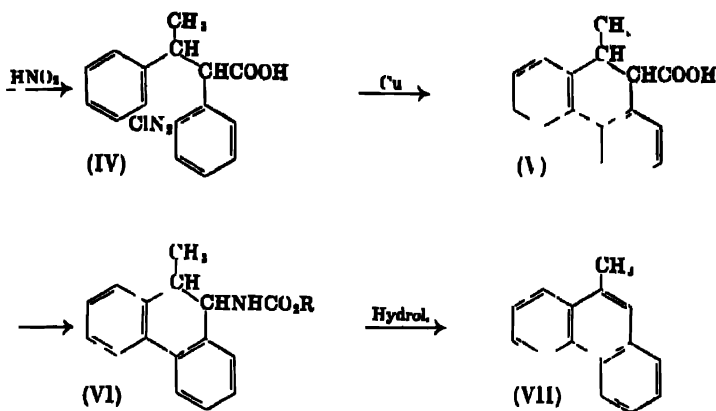
nated, and the amine (III) obtained on the hydrolysis of the cyclic amide was converted to the methyl dihydrophenanthrene carboxylic acid V

³ F. Mayer and Balle, *Ann*, 403, 167 (1914); see also Rapson and Robinson, *J. Chem. Soc.*, 1533 (1936).

⁴ F. Mayer and Englab, *Ann*, 417, 60 (1918).

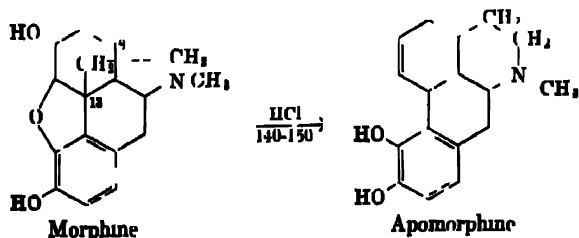
⁵ Windaus and Fickel, *Ber*, 57, 1871 (1924).

⁶ Windaus, H. Jensen and Schramme, *ibid.*, 57, 1875 (1924).



through the diazonium salt (IV). The ester of the acid was converted into the urethane, VI, and this was subjected to hydrolysis. An aminodihydrophenanthrene, which would be expected as the primary product, was not observed for it apparently loses the elements of ammonia at once and gives the hydrocarbon (VII) directly.

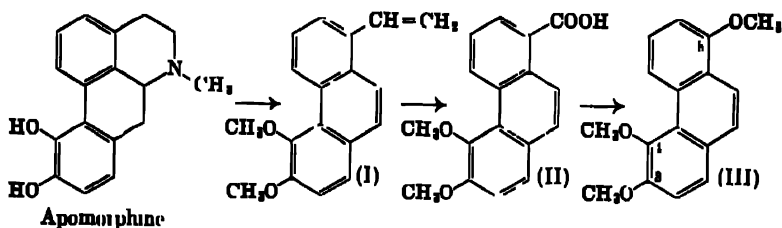
Apomorphine. In the years following Pschorr's initial discovery, his synthetic method proved to be of inestimable service in the investigation of the opium alkaloids. The complete characterization of morphol, morphenol, and the ethers of these compounds served to establish the positions of the three oxygen atoms in morphine, codeine, and thebaine and to indicate the nature of their respective states of combination. The method introduced by Pschorr was also of great assistance in elucidating some of the remarkable transformations of which these alkaloids are capable. One reaction of particular significance is the conversion of morphine by dehydration with acids into apomorphine:



The dehydration is accompanied by a molecular rearrangement, the ethanamine chain being displaced from C₁₁ to C₈. The oxide ring is also opened in the course of the reaction. The driving force for the change probably is derived from the tendency of the terminal ring to assume the aromatic condition after the dehydration to a dihydrobenzenoid nucleus.

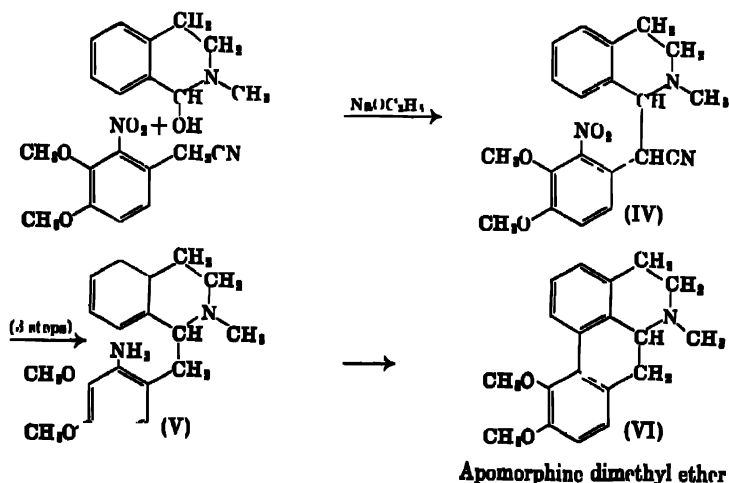
The transformation is a complicated one, and before the fact of a rearrangement was established it was the source of misleading inferences regarding the structure of morphine.

Pschorr was able to establish the structure of apomorphine with comparative ease by the application of the above methods of degradation and synthesis.⁶ On elimination of the nitrogen by successive exhaustive methylations a vinyl group was left as a residue of the original ethanamine chain. To determine the position of the vinyl group, and consequently the point of attachment of the nitrogen chain, the vinyl compound (I)



was oxidized to an acid (II) and this was converted by the Curtius method through the azide to the urethane and the corresponding amine. On hydrolysis of the diazonium salt and methylation, an ether (III) was obtained which was identified as 3,4,8-trimethoxyphenanthrene by its synthesis according to the standard method. The structure assigned to apomorphine on the basis of these results was fully confirmed by two independent syntheses of the dimethyl ether.

On the one hand, Avenarius and Pschorr⁷ condensed 3,4-dimethoxy-

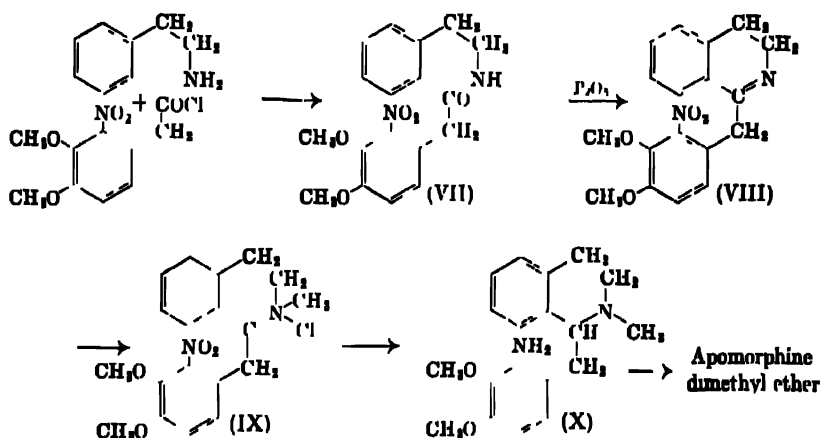


⁶ Pschorr and co-workers, *Ber.*, 35, 4377 (1902), 39, 3124 (1906); 40, 1954, 1965, 1998, 2001 (1907).

⁷ H. Avenarius and Pschorr, *ibid.*, 62, 321 (1929)

2-nitrobenzyl cyanide with α -hydroxy-N-methyltetrahydroisoquinoline, eliminated the nitrile group from IV by hydrolysis and decarboxylation, and reduced the nitro compound to an amine (V). The dihydrophenanthrene ring was then closed by the diazotization procedure of the regular Pschorr synthesis and the product (VI) was identified as apomorphine dimethyl ether.

Spath and Hromatka⁸ employed a method involving both a synthesis of the heterocyclic ring and a phenanthrene ring closure. 2-Nitrohomoveratryl chloride was condensed with β -phenylethylamine and the amide (VII) was submitted to cyclization with phosphorus pentoxide in boiling xylene solution (Bischler-Napieralski reaction) in order to produce the dihydroisoquinoline derivative (VIII). This was converted through the methiodide to the methochloride (IX), which on treatment with tin and hydrochloric acid was reduced to a tetrahydroisoquinoline with simultaneous reduction of the nitro group, giving X. The final phase of the



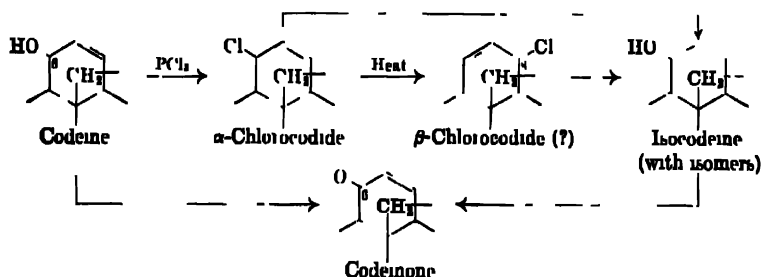
synthesis consisted in treating the diazotized amine with copper powder, as in Pschorr's general method. The ether was identified in the form of a benzoylation product.

The Structure of Morphine. Supposing the ring systems of morphine and of apomorphine to be the same, Pschorr suggested in 1902 a "pyridine-formula" for morphine corresponding to the structure eventually established for apomorphine, but the formula was not long sustained. In apomorphine the terminal carbon atom of the ethanamine chain is joined to the nucleus at C_8 , but in 1907 Knorr and Hörlein⁹ discovered evidence which definitely excludes this position as a point of attachment in the

⁸ Spath and Hromatka, *Ber.*, 62, 325 (1929).

⁹ Knorr and Hörlein, *ibid.*, 40, 3341 (1907).

case of codeine, and hence in the case of morphine. The investigations were concerned with pseudocodeine, one of three isomers of codeine which can be obtained from the chloride of this unsaturated secondary alcohol. The primary product resulting from the treatment of codeine with thionyl chloride, phosphorus trichloride, or phosphorus pentachloride is called α -chlorocodide. When α -chlorocodide is heated with hydrochloric acid or when it is heated above the melting point in an indifferent solvent it is converted into an isomer, β -chlorocodide. The two chlorides on hydrolysis yield mixtures of isomeric alcohols, one of which, isocodeine, is known to be the epimeric form of codeine, the isomerism being due to a different spatial arrangement of the hydroxyl and hydrogen at C_6 . The relationship is indicated in the formulas with the use of full and dotted lines to represent a projection of the substituent on one or the other side of the plane of the ring. That codeine and isocodeine differ only with regard to the configuration at C_6 is fully established by the fact that both sub-



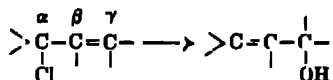
stances yield the same ketone, codeinone, on oxidation. Knorr definitely established the presence in codeinone of an oxygen atom at C_6 by degradation to 3,4,6-trimethoxyphenanthrene.

Although there is opportunity for similar isomerism in the case of the chlorocodides, it is probable that β -chlorocodide is not the epimeric form of the α -compound but a structural isomer in which the chlorine atom is located at C_3 .¹⁰ The changes evidently are complicated, but for the present it will be sufficient to consider the products resulting from the hydrolysis of the two chloro compounds.

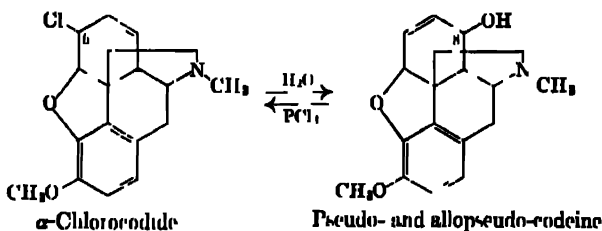
The halogen atom of α -chlorocodide, and to a lesser extent the chlorine atom of β -chlorocodide, shows a degree of reactivity reminiscent of that of allyl chloride, and these chlorocodides can be hydrolyzed by prolonged boiling with dilute acids. Each substance gives a mixture, in different proportions, of the alcohols isocodeine, pseudocodeine, and allo pseudocodeine. Pseudocodeine and allo pseudocodeine are epimers, for they yield

¹⁰ Schöpf and Hirsch, *Ann.*, **489**, 224 (1931); L. F. Small, "Chemistry of the Opium Alkaloids," pp. 362-363.

the same ketone on oxidation. The oxidation product is known as pseudo-codeinone, and it is a structural isomer of codeinone. From the degradation of pseudocodeinone Knorr and Hörlein obtained an ether identical with synthetic 3,4,8-trimethoxyphenanthrene, proving that the carbonyl group is located at C₈. It is evident that a structural change occurs in the formation of pseudocodeine and allopseudocodeine, but that this does not involve the ring system is shown by the fact that pseudocodeine can be converted to both α - and β -chlorocodide. The first adequate interpretation of the reaction was advanced independently in 1923 by Gulland and Robinson¹¹ and by Wieland and Koralek.¹² It was suggested that the change is due to an α,γ -shift, as in an ordinary allylic rearrangement:



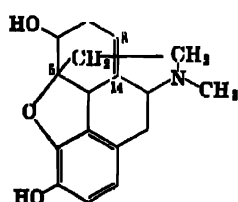
According to the later (1925) views of Gulland and Robinson, the reaction is represented as shown in the formulas:



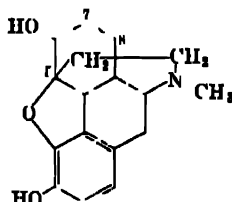
If the 8-position is involved in this reversible shift it is not available as a seat of attachment for the ethanamine chain as pictured by Pschorr. Finding Pschorr's hypothesis inconsistent with their experimental observations, Knorr and Hörlein proposed an alternate formula in which the chain is joined to the nucleus at C₇. Although this representation occupied a position of considerable prominence in the discussions which followed, it did not account adequately for the codeine-pseudocodeine change. The formula was later modified by Wieland by transferring the double bond from C₅-C₁₄ to C₇-C₈, in recognition of the presence of an allylic grouping. The same feature is embodied in the formula of Gulland and Robinson and the only distinction between this and the Wieland formula is in the location of the C. C. N. chain.

¹¹ Gulland and R. Robinson, *J. Chem. Soc.*, 123, 980 (1923).

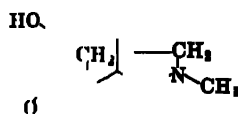
¹² Wieland and Koralek, *Ann.*, 433, 267 (1923).



Knorr-Horlein (1907)

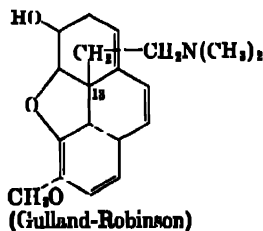
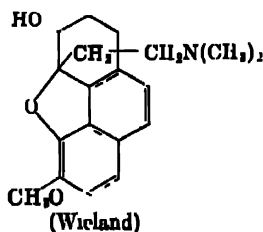


Wieland (1925)



Gulland-Robinson (1925)

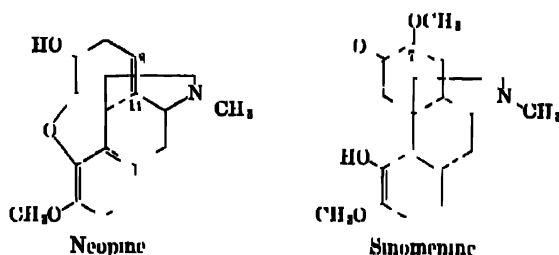
From the brief review which has been given of the main lines of evidence in the case it can be seen that the last two formulas represent the only structures possible for the alkaloid. The positions of the three oxygen atoms are fixed by the degradations to phenanthrene derivatives, while the attachment of the nitrogen atom at C_9 is established by the relationship to apomorphine. The isolated double bond must be in an α,β -position with respect to the secondary alcoholic group, and in view of the evidence that the alcoholic group of pseudocodeine is at C_8 , the position C_7-C_8 is definitely indicated. The ethanamine chain cannot be located at C_{14} , because this carbon atom is capable of becoming unsaturated (thebaine, β -methylmorphimethine), and the only positions available are C_7 and C_{11} . Although an entirely rigid distinction between these two possibilities has not been made, there are cogent reasons for regarding the Gulland-Robinson formulation as the expression of the true structure. One argument is that the ready extrusion of the side chain with the aromatization of the unsaturated ring finds an explanation in this formula but not in that of Wieland. Further support is found in the isomerization of α -methylmorphimethine to β -methylmorphimethine, a change which is known to involve the migration of a double bond to a position of conjugation. If the Wieland formulation were correct, the unsaturated linkage would be expected to migrate into the central nucleus and β -methylmorphimethine should be a naphthalene derivative. The

 β -Methylmorphimethine

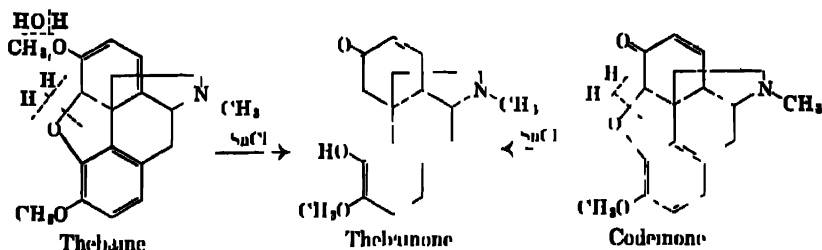
properties, however, are that of a more highly unsaturated compound, and the alternate formulation provides an adequate explanation: the

side chain at C_{13} blocks a further migration of the double bond into the unsaturated central ring. The evidence,¹³ although not entirely conclusive, has led to the general acceptance of the formula of Gulland and Robinson.

Related Alkaloids. Closely related in structure to morphine, codeine, and thebaine are the alkaloids neopine,¹⁴ isolated in small amounts from opium, and sinomenine,¹⁵ obtained from a woody vine (*Sinomenium acutum*) of Eastern Asia. Neopine differs from codeine only in the position of the double bond in the terminal hydroaromatic ring, as shown by the



fact that on hydrogenation it yields dihydrocodeine. The location of the double linkage in question at C_8-C_{14} is established by the formation of β -methylnorphimethine (page 26) on the decomposition of neopine methohydroxide. Sinomenine is closely related to thebainone, an unsaturated ketone obtained by the action of stannous chloride on either thebaine or codeinone, the relationship being that sinomenine can be converted into the optical antipode of dihydrothebainone. Sinomenine evidently belongs to a stereochemical series different from that of the alkaloids of the morphine group, and the Japanese investigators Kondo and Goto have established the presence of a methoxyl group at C_7 by a degradation to 2,6-dimethoxy-3,5-dihydroxyphenanthrene. They regard the alkaloid as *d*-7-methoxythebainone and formulate it in terms of the Gulland-Robinson conception of the structure of thebainone.

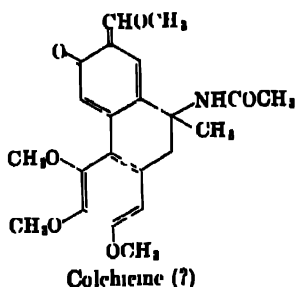


¹³ See also, Rensch, *Ann.*, **452**, 211 (1927).

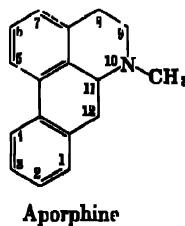
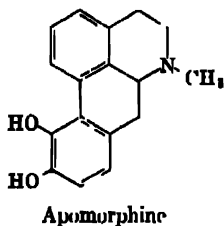
¹⁴ I. F. Small, "Chemistry of the Opium Alkaloids," p. 205.

¹⁵ Kondo and Ochiai, *Ann.*, **470**, 224 (1929), *Ber.*, **63**, 616 (1930), Goto, *Ann.*, **485**, 247 (1931).

Among the other alkaloids of *Sinomenium acutum*, disinomenine appears to be a dimolecular product resulting from the dehydrogenation of the phenolic substance sinomenine.¹⁶ Diversine, from the same source, has been shown by zinc dust distillation experiments to contain the phenanthrene nucleus.¹⁷ Similar evidence is available regarding porphyroxine,¹⁸ an opium alkaloid for which the structure of an endocarbonyl dihydrocodeine has been tentatively suggested. Colchicine¹⁹ is also a phenanthrene alkaloid, for it has been degraded to a tetramethoxymethylphenanthrene and to 9-methylphenanthrene. According to the formula provisionally considered by Windaus the substance bears some points of similarity to the morphine alkaloids.



The Aporphine Group. Although the conversion of morphine into apomorphine proved to be of less significance in elucidating the structure of the alkaloid than was at first expected, the product of the rearrangement reaction has acquired a position of considerable importance in providing a connecting link between the bases of the morphine series and a number of other phenanthrene alkaloids derived from the parent sub-



stance aporphine. As the formulas indicate, apomorphine is the 3,4-dihydroxy derivative of aporphine. The parent compound does not occur in

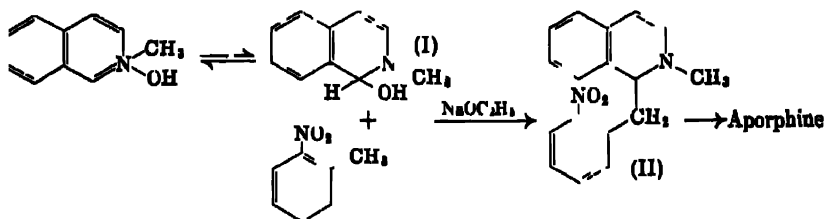
¹⁶ Goto and Kitasato, *Ann.*, 481, 81 (1930).

¹⁷ Kondo and Nakajima, see *Chem. Zentr.*, 1, 1839 (1927).

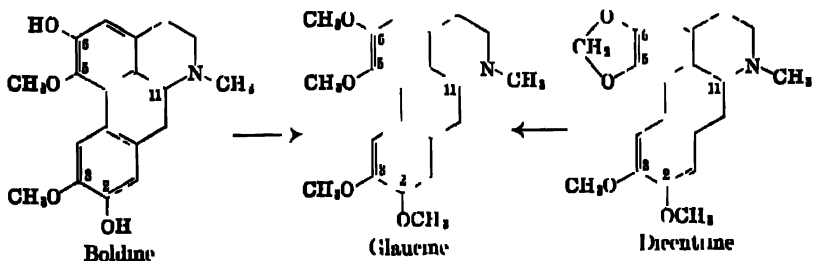
¹⁸ L. F. Small, "Chemistry of the Opium Alkaloids," pp. 207-209.

¹⁹ Windaus, *Ann.*, 439, 59 (1924)

nature but it has been synthesized by Gadamer²⁰ from *o*-nitrotoluene and the pseudo base (I) of *N*-methylisoquinolinium hydroxide. After reduction of the nitro group of II and saturation of the double bond, the ring was closed by the Pashorr method.



The alkaloids of the aporphine series are all very similar to one another and the majority of them are more or less highly etherified derivatives of either 2,3,5,6- or 3,4,5,6-tetrahydroxyaporphine. Boldine, glaucine, and dicentrine belong in the former class and they are known to be related not only in structure but in the configuration at the asymmetric center C_{11} . Boldine on methylation yields a product identical with natural glau-



cine,²¹ and the same substance has been obtained from dicentrine by hydrolysis of the methylenedioxy group and methylation of the resulting 2,3-dimethoxy-5,6-dihydroxy compound.²² The cleavage of the methylene ether linkages without disturbing the methoxyl groups is accomplished by warming the compound with dilute sulfuric acid and phloroglucinol.²³ The formaldehyde split from the one molecule forms a red condensation product with phloroglucinol. The structure of glaucine, the key substance of the group, was established by Gadamer's synthesis²⁴ of the alkaloid

²⁰ Gadamer, Oberlin and Schoeler, *Arch. Pharm.*, **263**, 81 (1925)

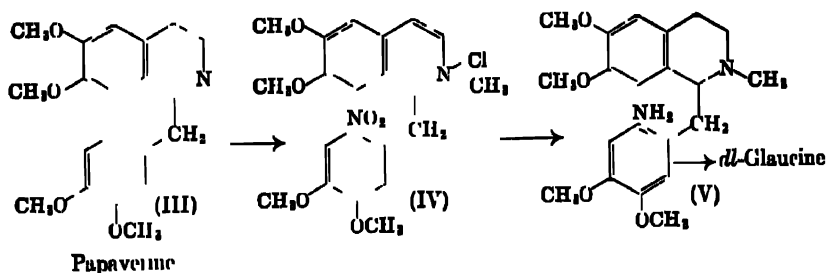
²¹ Warnat, *Ber.*, **58**, 2768 (1925), **59**, 85 (1926)

²² Otsu, *J. Pharm. Soc. Japan*, **48**, 85 (1928) [*Chem. Zentr.*, **2**, 672 (1929)]

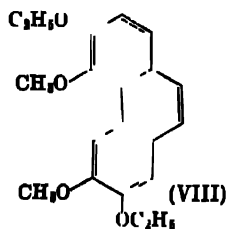
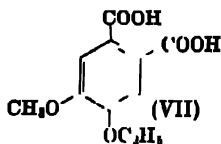
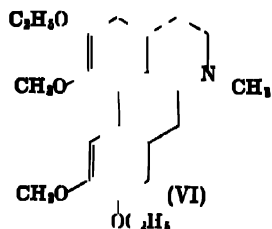
²³ Späth and Quastenberg, *Ber.*, **60**, 1392 (1927)

²⁴ Gadamer, *Arch. Pharm.*, **249**, 680 (1911)

from papaverine, a method suggested by the hypothesis that this represents the course of the phytosynthesis. The process already had been carried nearly to completion by Pschorr.²⁵ The essential steps consist in the nitration of papaverine (III), reduction of nitropapaverine methochloride (IV) to aminotetrahydro-N-methylpapaverine (*dl*-aminolaudanose), V, and ring closure through the diazonium salt.



dl-Dicentrine has been synthesized by a similar method and the naturally occurring *d*-base obtained by resolution.²⁶ The structure of boldine was established by the application of the standard methods which have been developed for the investigation of partially alkylated members of the series. Having established the positions of the oxygen atoms by the conversion to glaucine, Warnat²¹ sought to locate the methoxyl groups by oxidation. If the two protected groups had been in the same ring they might have appeared in a benzene di- or tri-carboxylic acid, but apparently both of the terminal rings were destroyed in the process for only oxalic acid was obtained. Warnat's conclusion that each nucleus contains a phenolic group was later confirmed by Schlittler,²⁷ who oxidized boldine diethyl ether (VI) and obtained the methyl ethyl ether of nor-*m*-hemi-



pinic acid (VII). On account of the symmetry of the acid VII, this observation was not sufficient to establish the positions of the groups in the

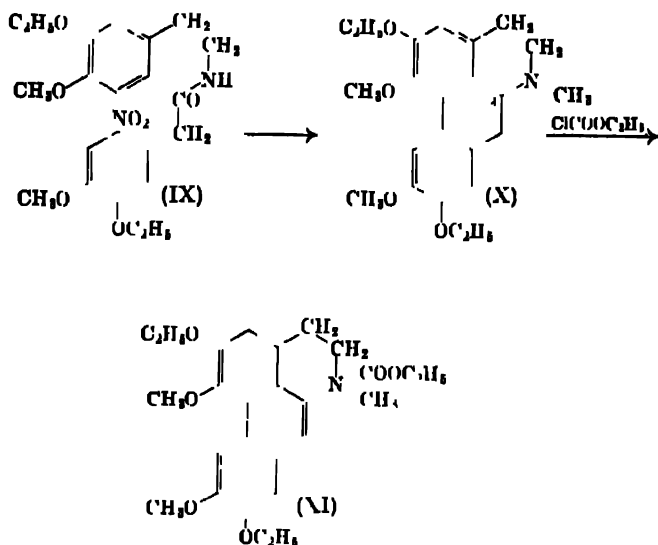
²⁵ Pschorr, Stahlin and Silberbach, *Ber.*, 37, 1926 (1904)

²⁶ R. D. Haworth, W. H. Parkinson and J. Rankin, *J. Chem. Soc.*, 2018 (1923), 29 (1926)

²⁷ Schlittler, *Ber.*, 66, 988 (1933)

ring in question, for the same acid would result if the methoxyl and ethoxyl groups were interchanged.

To complete the evidence Schlittler chose the synthetic approach, while Späth and Tharrer²⁸ independently and at the same time undertook the Hofmann degradation of the diethyl ether VI. This ether, which is prepared with the use of diazoethane, was converted in succession to a methine base, to an alkoxyvinylphenanthrene, to an alkoxyphenanthroic acid, and finally to the dimethoxydiethoxyphenanthrene VIII, which was identified by synthesis according to the P-chorr method. Schlittler's synthesis, employing the amide IX, involved a Bischler-Napieralski isoquinoline ring closure, N-methylation, and a phenanthrene ring closure. The final product, X, was a racemic mixture and a comparison with boldine diethyl ether was made by the convenient method introduced by Gadamer.²⁹ On

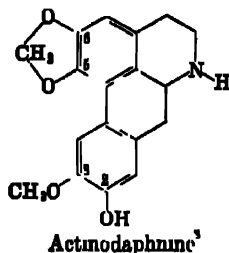
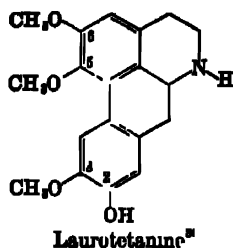


reaction with ethyl chloroformate the nitrogen ring is cleaved and the center of asymmetry destroyed. The ester XI proved to be identical with the material prepared from the natural alkaloid.

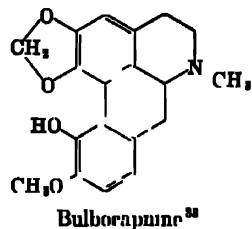
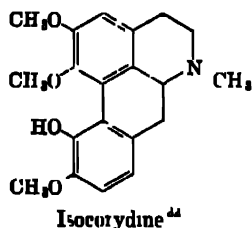
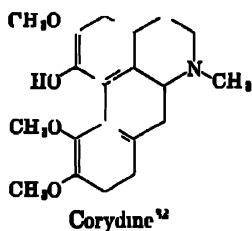
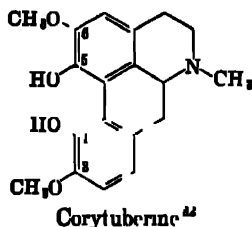
Similar methods of investigation led to the elucidation of the structures of two additional members of the 2,3,5,6-substituted series, laurotetanine and actinodaphnine. These alkaloids are secondary bases, and since they lack the N-methyl group characteristic of aporphine they are regarded as derivatives of noraporphine

²⁸ Späth and Tharrer, *Ber.*, **66**, 904 (1933)

²⁹ Gadamer and Knock, *Arch. Pharm.*, **259**, 135 (1921)



Four of the alkaloids of corydalis have been found to be alkyl derivatives of 3,4,5,6-tetrahydroxyaporphine. The structures are shown in the accompanying formulas.



Two members of the aporphine group have been found by Barger and Girardet²⁶ to contain but three atoms of oxygen. Pukateine²⁵ has the structure of 4-hydroxy-5,6-methylenedioxyaporphine, while laureline²⁶ is 3-methoxy-5,6-methylenedioxyaporphine. A related alkaloid laurepukine²⁷ is either 3,4-methylenedioxy-5,6-dihydroxyaporphine or the

²¹ Structure Späth and Straubel, *Ber*, 61, 2495 (1928) Späth and Tharrer, *Ber*, 66, 583 (1933), Barger and Fülherschmidt, *J Chem Soc*, 2914 (1929), Barger, J Eisenbrand, L. Eisenbrand and Schlittler, *Ber*, 66, 450 (1933)

²² Structure Ghose, Krishna and Schlittler, *Helv Chim Acta*, 17, 919 (1934)

²³ Structure Späth and Berger, *Ber*, 64, 2038 (1931) Synthesis of corytuberine dimethyl ether Späth and Hromatka, *ibid*, 61, 1692 (1928)

²⁴ Structure Gadamer, *Arch Pharm*, 249, 475, 503 (1911) Gadamer and Kuntze, *ibid*, 249, 598, (1911) Synthesis of the methyl ether Gulland and R. D. Haworth, *J Chem Soc*, 1132 (1923)

²⁵ Barger and Girardet, *Helv Chim Acta*, 14, 481 (1931) See also Goto, *Ann*, 521, 175 (1935)

²⁶ Synthesis of l-pukateine methyl ether. Barger and Schlittler, *Helv Chim Acta*, 15, 381 (1932)

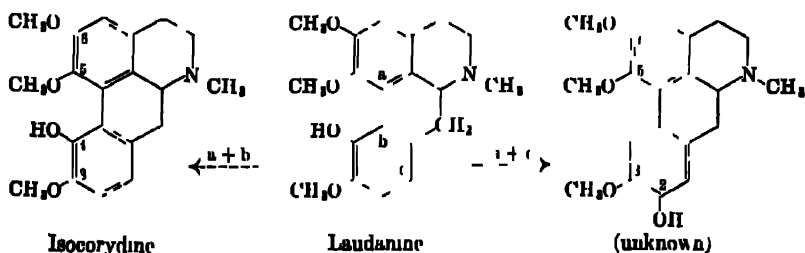
²⁷ Synthesis Schlittler, *ibid*, 15, 394 (1932)

²⁸ Girardet, *ibid*, 14, 504 (1931)

3,4-dihydroxy-6,7-methylenedioxy compound; by analogy with all of the other aporphines, the former structure appears to be the more probable. Domesticine⁵⁸ contains one phenolic group, a methoxy, and a methylenedioxy group, but the structure has not been completely elucidated.

While the synthetic preparation of ethers of the alkaloids has been of great value in solving problems of structure, it would be of further interest to produce partially alkylated compounds of the type found in nature. With this end in view, the direct synthesis of phenolic aporphines is being investigated by both Japanese and English workers.⁵⁹ The hydroxyl groups are best protected during the synthesis by means of the benzyl group, for this can be removed under such mild conditions of hydrolysis (concentrated hydrochloric acid at 50°) that methoxyl groups in the molecule remain intact.

Genetic Relationships. One of the most striking outcomes of the investigations in the field of the alkaloids is the recognition of many close structural relationships between individual members of the broad group. The interrelationships among the various phenanthrene alkaloids of opium and among the aporphine alkaloids of corydalis have already been indicated, but it is possible also to discern close associations between different groups. That the benzylisoquinoline alkaloids, papaverine, laudanine, laudanoline, and narcotine are of a molecular pattern very similar to that of the bases of the aporphine group is clearly shown by the syntheses discussed above, for example, the synthesis of glaucine from papaverine. A still closer relationship is evident in the formulas of isocorydine, laudanine, and laurotetanine. If the two aromatic nuclei of laudanine were linked by dehydrogenation at positions *a* and *b*, the product would have the structure of isocorydine. The ortho position *c* is also available (rotation of the lower ring), and a ring closure between positions



a and *c* would lead to a 2,3,5,6-substituted aporphine, N-methyl-laurotetanine (or 2-demethylglaucine). The exact correspondence in the loca-

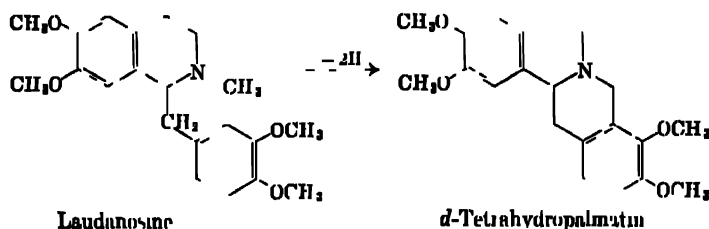
⁵⁸ Kitamoto, *Chem. Zentr.*, 1, 105 (1927), 2, 1035 (1927), Onada, *ibid.*, 2, 672 (1928).

⁵⁹ Kondo and Ishiwata, *Ber.*, 64, 1533 (1931), Gulland, Ross and Smellie, *J. Chem. Soc.*, 2885 (1931), Douglas and Gulland, *ibid.*, 2895 (1931).

tion of the methyl groups in the first case may be only a coincidence, but the above relationship unquestionably accounts for the preponderance of aporphine alkaloids with substituents at either the 2,3,5,6- or the 3,4,5,6-positions.

It is interesting that the hypothetical ring closures pictured above are not without analogy. It will be recalled that Erdtmann (page 6) succeeded in effecting a phenanthrene ring closure by the oxidation of a tetrahydroxydibenzyl of structure quite similar to that of laudanine. It will be observed in the formulas above that the positions *a*, *b*, and *c* are either ortho or para to an activating hydroxyl or methoxyl group, as in Erdtmann's compound. The union of two phenolic nuclei by oxidation is a well-known process, although aromatic ethers do not exhibit such a reaction. Considered from this point of view it would appear probable that in the course of the phyto-synthesis methylation occurs subsequent to the establishment of the aporphine ring system.

It should be observed that the benzyliisoquinoline alkaloids theoretically can undergo intramolecular cyclization in still a different direction, as suggested by the following formulas:



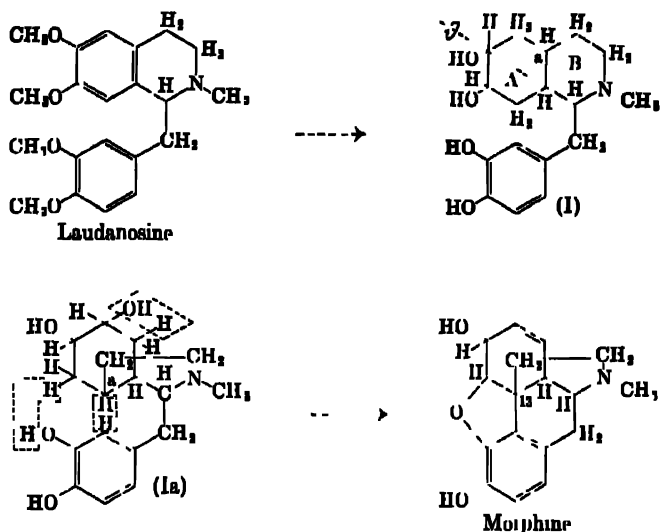
The ring system of *d*-tetrahydropalmatin is characteristic of corydalme, the principle alkaloid of the corydalis plants, and of berberine, an important constituent of plants of the genus *Berberis*.

Of some twenty-three alkaloids which have been isolated from opium, five belong to the morphine group and the remainder are either benzyliisoquinoline derivatives or simpler substances related to the latter alkaloids as either building units or by-products of the phyto-synthesis. A relationship between the two principal groups may be inferred both because the substances occur together and because morphine can be correlated with the benzyliisoquinoline alkaloids through apomorphine and the aporphines. A closer relationship has been suggested by Gulland and Robinson⁴⁰ and by Schöpf,⁴¹ who picture the benzyliisoquinoline alkaloids as the natural precursors of the morphine compounds. Laudanosine, for example, may be supposed to become hydrogenated in the isoquinoline

⁴⁰ Gulland and R. Robinson, *Mém. Proc. Manchester Lit. Phil. Soc.*, **60**, 79 (1923)

⁴¹ Schöpf, *Ann.*, **452**, 211 (1927).

nucleus and demethylated, except for the N-methyl group. If, in the hypothetical formula I, ring A is revolved through an angle of 180° about



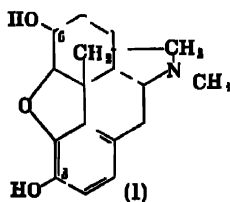
the axis of the dotted line, the formula can be written as in Ia, ring B being distorted from the hexagonal arrangement. Ring closure is imagined as taking place at the carbon atom (a) of an original bridge head and this consequently is the point of attachment (C_{17}) of the ethanamine chain in the final product. In this way it is possible to conceive the phyto-synthesis of the complex ring system of morphine from benzyloquinoline alkaloids or their progenitors by a process involving no readjustment of the carbon skeleton.⁴²

The Problem of Drug Addiction. Certain narcotic drugs, among which morphine and cocaine are the outstanding examples, possess invaluable medicinal qualities combined with the insidious property of addiction. Morphine is valued chiefly for its powerful analgesic action (deadening of pain), but even where it is possible to control the use of the drug to legitimate needs for medicinal purposes the continued use of morphine in cases of recurring illnesses is fraught with great danger on account of its ability to produce progressively increasing tolerance and because of the distressing abstinence symptoms arising when it is withheld from a tolerant individual. The cocaine problem has been in large part solved, at least from the point of view of medicine, through the substitution of the synthetic substance procaine, an effective local anes-

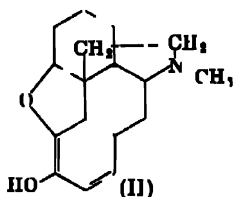
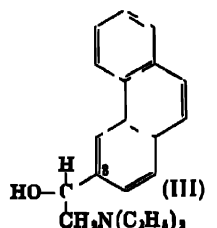
⁴² An alternate hypothesis which assumes a molecular rearrangement has been discussed by R. Robinson and Suganawa, *J. Chem. Soc.*, 3163 (1931)

thetic having little or no addiction properties. In the hope of providing a means of effectively combatting morphine addiction in a similar manner, a series of investigations of the chemistry and pharmacology of the opium alkaloids was initiated in this country in 1929. Cooperative research on the problem is being carried on under the National Research Council at the Universities of Virginia and Michigan in conjunction with various branches of the government service.⁴³ The primary object of the work is to find a drug or drugs capable of replacing morphine in some of its uses in medicine without causing addiction.

The chemical research group at Virginia, headed by L. F. Small, has followed two main lines of attack, the modification of the morphine molecule (Small, Lutz), and the synthesis of simpler compounds having various features of the morphine structure (Mosettig, Burger, van de Kamp).⁴⁴ Prior to this work it was well known that codeine, the phenolic methyl ether of morphine, has a comparatively low addiction property, and although the drug is less powerful than morphine in analgesic action it can replace the more dangerous drug in many instances if used in large doses. A comprehensive study of a number of phenol-phenol ether pairs which were prepared by the Virginia group and tested pharmacologically at the Michigan laboratories has definitely shown that the presence of the free phenolic group at C₃ is important for the depressant and analgesic action of morphine as well as for its effect on respiration. Conversely,



Morphine

Dihydrodesoxy-
morphine-DDiethylaminomethyl-
3-phenanthryl carbinol

the alcoholic hydroxyl group at C₃ appears to be inhibitory to these actions of morphine. Methylation or acetylation⁴⁵ of this hydroxyl results in increased activity, and the effect is still greater when the group is completely eliminated, as in the desoxymorphines. One member of the series, dihydrodesoxymorphine-D (II), exerts an analgesic action over ten times greater than that of morphine and the general depressant effect is 30-40 times as great. Since the compound is only about three times as

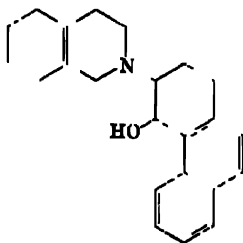
⁴³ White, *Science*, 123, 97 (1931)

⁴⁴ Edmunds, Eddy and Small, *J Am Med Assoc*, 103, 1417 (1934)

⁴⁵ Eddy and Howe, *J Pharmacol*, 53, 430 (1935)

toxic as morphine it may well be of value in medicine and the substance has been submitted to clinical trial.

How much of the morphine ring system is important to its action is still a matter of conjecture, but it has been established that the opening of the ethanamine ring, as for example in α -methyldorphimethine, results in a great loss of the typical morphine effects. Although the complex ring system is still beyond reach by synthesis, the work on comparatively simple derivatives of phenanthrene and diphenylene oxide nevertheless has yielded encouraging results. 3-Phenanthrol is mildly depressant and displays some degree of analgesic action. A second phenolic group, particularly when located at C₆, further strengthens the actions. The open-chain aminohydroxy compound III exhibits very typical morphine-like effects. With all of the isomeric substitution products of phenanthrene which have been tested the activity is greatest when the attachment is made at the 3-position. Among the synthetic products so



far obtained the highest analgesic action is exhibited by 3-(1,2,3,4-tetrahydroisoquinolino)-4-hydroxy-1,2,3,4-tetrahydrophenanthrene.⁴⁶ The compound is in some ways nearly as potent as codeine and pseudocodeine, the effective analgesic dose being 15 mg. per kg., as compared with 10 mg. per kg. for codeine. Further developments in the field will be awaited with great interest.

At the beginning of the work on the problem progress on the synthetic side was retarded by the necessity of developing the fundamental chemistry of the substitution products of phenanthrene. With many lines of investigation now pointing to phenanthrene as a fundamental structural unit of prime importance to natural products exhibiting a wide variety of physiological effects, the rapid advancement in the chemistry of the hydrocarbon can be looked for in the near future. Unlike its isomer, phenanthrene has found little use as the basis for dyes and such few investigations of the hydrocarbon as have been made since its discovery in 1872 have been prompted for the most part by interest in natural products. For example, A. Werner's studies of sulfonation and

⁴⁶ Mosettig and Burger, *J. Am. Chem. Soc.*, 57, 2189 (1935).

J. Schmidt's studies of nitration were instigated with the object of preparing morphol from the hydrocarbon. Each of the important phenanthrene syntheses had its origin in considerations similar to those which prompted Paschorr to develop his classical method. With the interest in phenanthrene chemistry enhanced by the many important discoveries in the period 1930-1935, the long neglected hydrocarbon has acquired a position of new significance and of major importance.

Chapter II

Resin Acids

The exudations of pine and fir trees are composed mainly of two types of materials, acidic resins or resin-forming substances, and essential oils. The fresh, limpid secretion from the tree is a solution of the former substances in the oils and is known as an oleoresin or crude turpentine. On aging, the more volatile oils evaporate, some oxidation occurs, and the material forms a viscous gum and then, if in the form of a thin film, a hard, glass-like resin. Substances of this type, which contain the bulk of the acidic constituents in modified form, are often found as fossil resins after the plant has decayed. The copal and kauri copal of various tropical trees are obtained largely as fossil resins. The most abundant resins, however, are those obtained from living trees. The essential oils are removed from the oleoresins or gum resins by steam distillation, the distillate affording oil of turpentine. This consists principally of a mixture of terpene hydrocarbons. The non-volatile part forms a hard, resinous mass of varying degrees of color. The most plentiful and commercially important resin prepared in this way is the rosin, or colophony, obtained along with oil of turpentine from American pines. By suitable treatment, rosin can be converted in good yield into the substance known as abietic acid (*L. abies*, fir tree), the best known of the resin acids.

Abietic acid is not an original constituent of the tree secretions and it apparently is not present as such to an appreciable extent in rosin. It arises as a transformation product of the natural, primary acids in the course of the preparation and further treatment of rosin, possibly through a succession of intermediate forms. Rosin appears to be a mixture of acids in various stages of transformation. The original oleoresin acids are very sensitive to the action of mineral acids and to heat, and they are easily oxidized by the air. Changes occur during storage and under the influence of the mild heat treatment involved in the steam distillation of the essential oils from the crude gum turpentine. It is not an easy matter to determine if a substance isolated even under the mildest conditions is a primary constituent, but a number of different acids apparently belonging to this category have been characterized. Nearly all of these substances have the same composition as abietic acid, namely $C_{19}H_{29}COOH$, and are convertible into abietic acid under the influence

of heat or on treatment with acids.¹ Examples of "sapinic" acids which are easily converted into abietic acid are the "aleppic" and "pinic" acids (m.p. 148° and m.p. 120°) obtained by Dupont² from *Pinus halepensis* and *Pinus pinea*. In the same class is the l-pimaric acid (m.p. 152°, $[\alpha]_D -282^\circ$) from *P. excelsa*,^{1,3} from the American *P. palustris*,⁴ and from French galipot,⁵ the sirupy, semisolid oleoresin from cluster pine (*P. pinaster*). l-Pimaric acid is isomerized to abietic acid by hot glacial acetic acid. The amount obtained from galipot is less than two per cent of the total, and all of the primary resin acids appear to occur in admixture with several other isomeric substances. Both the isolation and the characterization of the original acids is complicated by their tendency to isomerize and by their sensitivity to oxidation. A small amount of a neutral, vaguely characterized companion of the acids known as "resene" appears to catalyze the oxidation.⁶ Because of these difficulties, and probably also because of the tendency of the closely related substances to form mixed crystals, many of the early reports are subject to question and the information regarding the primary constituents of the resins is still very incomplete (see page 68).

The Preparation of Abietic Acid from Rosin. The conversion of rosin into abietic acid was at one time thought to involve a hydration. Solutions of rosin in aqueous alcohol or acetic acid when prepared at moderate temperatures deposit no crystals of abietic acid and do not respond to precipitation tests for this substance, but a considerable amount of abietic acid can be obtained after the solutions have been boiled for some time. This was regarded as an indication that rosin consists principally of the anhydride of abietic acid,⁷ but the true anhydride is quite different in properties from rosin,⁸ and there is no evidence that the water present in the solvents enters into the reaction. Ruzicka and Meyer⁹ perfected an earlier method¹⁰ of preparing abietic acid which is directly contradictory to the hydration theory. They distilled rosin under high vacuum (at pressures less than 1 mm) at 200-210° and obtained a glassy distillate (90%) which crystallized readily from alcohol. It appears that the conversion of rosin into abietic acid involves only an isomerization.

The isomerization of rosin can be accomplished both by heating and by treatment with acids, but in each case there is danger of carrying the

¹ Dupont, *Bull. soc. chim.*, [4], 29, 718 (1921)

² *Ibid.*, [4], 35, 879 (1924), Dupont and Dubourg, *ibid.*, [4], 36, 1029 (1926)

³ Dupont, *Compt. rend.*, 172, 1378 (1921)

⁴ Palkin and Harris, *J. Am. Chem. Soc.*, 55, 3677 (1933)

⁵ Ruzicka, Balas and Vilim, *Helv. Chim. Acta*, 7, 458 (1924).

⁶ Kessler, Lowy and Faragher, *J. Am. Chem. Soc.*, 49, 2898 (1927)

⁷ Knecht and Hibbert, *J. Soc. Dyers and Colour.*, 35, 150 (1919)

⁸ Fonrobert, *Chim. Umarches Fette, Oels, Wachsen Harze*, 36, 373 (1929)

⁹ Ruzicka and J. Meyer, *Helv. Chim. Acta*, 5, 315 (1922)

¹⁰ Easterfield and Bagley, *J. Chem. Soc.*, 35, 1238 (1904).

process too far. Abietic acid is more stable than the primary acids of the oleoresins, but it in turn can be converted into isomeric substances ("pyroabietic acids") characterized in general by their positive rotation, in contrast to the levorotary abietic acid, and by higher melting points ($5-10^\circ$). Ruzicka and Meyer⁹ found that the acids produced by isomerization of abietic acid at 300° and by treatment with acetic and hydrochloric acids still contain two double bonds. The further changes probably are due to stereochemical rearrangements or to migrations of the double bonds.

The fact that abietic acid represents only a relatively stable phase in a long series of isomerizations has been a source of much confusion. Some samples of the acid prepared from rosin probably represent more extensively isomerized products than others. The problem of identification is a difficult one and the melting point alone does not provide a very reliable criterion of purity. The melting point of abietic acid is not sharp and some decomposition may occur at the melting temperature. A further complication is that the melting points of the pyroabietic acids are only slightly above that of very pure abietic acid. A high melting point may indicate secondary isomerization rather than a great purity of the sample. The identity of preparations from different species of pines is still subject to uncertainty. In order to compare the "pinabietic acid" from a liquid resin resulting from the sulfite wood process in Scandinavia and Finland with abietic acid from rosin, Aschan and Levy¹¹ undertook a joint study of the products of oxidative degradation, but the results were inconclusive.

The methods of preparing abietic acid from rosin which are regarded as being the most reliable are those employing the mildest conditions for the isomerization. Steele's¹² method is both reliable and convenient, and it has been much employed in the studies of structure. Rosin of good quality (not oxidized) is dissolved in 98% acetic acid and the solution is boiled for two hours, filtered, and cooled. Abietic acid separates in crystalline condition and is obtained in a fairly pure state and in about 40% yield after one recrystallization from acetic acid. Ruzicka¹³ adopted the Steele method after finding that material prepared in this way (m.p. $159-161^\circ$, $[\alpha]_D^{25} -77^\circ$) was entirely comparable with that obtained by high vacuum distillation. Another method¹⁴ depends upon the formation of a crystalline acid salt of the composition $C_{19}H_{29}COONa \cdot 3C_{19}H_{29}COOH$ (m.p. 175°). This complex is sparingly

¹¹ Aschan and Levy, *Ber.*, **60**, 1923 (1927)

¹² Steele, *J. Am. Chem. Soc.*, **44**, 1333 (1922).

¹³ Ruzicka and Schinz, *Helv. Chim. Acta*, **6**, 662 (1923).

¹⁴ Dupont, Desalbre and Bernette, *Bull. int. du Pm*, No. 22, 340 (1926).

soluble in alcohol, whereas the normal sodium salt dissolves easily in this medium and does not tend to crystallize. The acid salt provides a convenient means of isolating abietic acid, but it is necessary first to isomerize the rosin in order to obtain a precipitation.⁶ In the method of Dupont,¹⁴ and in the more recent modified procedure of Palkin and Harris,¹⁵ rosin is isomerized by boiling a solution of the material in alcohol with hydrochloric acid. After the addition of the proper amount of sodium hydroxide the acid sodium salt separates in a crystalline condition. The free acid, m.p. 170-174°, $[\alpha]_D -102^\circ$, is easily obtained in good yield in this way, but it is doubtful if the material is identical with that produced by the methods described above. The mineral acid may produce secondary isomerizations.

The substances of the abietic acid group are among the most abundantly available and the cheapest of organic acids. Rosin is an important article of commerce, the bulk of the world's supply coming from this country. The rosin produced in the United States has been valued at about forty million dollars per annum, about 60% of the material being exported. The crude sodium salt, "sodium resinate" is used for sizing paper and it is also employed as an admixture in the manufacture of yellow laundry soap, the aqueous solution being colloidal and having detergent properties. The liquid esters of abietic acid, including the glyceryl esters, are useful as plasticizers and softeners in the manufacture of nitrocellulose lacquers. Manganese and cobalt resinates, consisting essentially of the normal, benzene-soluble salts of abietic acid, are important driers for paint oils and varnishes. Rosin is also used as a flux in soldering, for rosining violin bows, as a caulking material, etc.

The Preparation of *d*-Pimaric Acid. It was stated above that French galipot, chiefly from *P. pinaster (maritima)*, contains an unstable primary acid, *l*-pimaric acid, which is convertible into abietic acid. Galipot also is the source of another acid which is isomeric with *l*-pimaric acid but which is entirely distinct from this substance and from all of the acids of the abietic acid group. The substance is dextrorotatory and is known as *d*-pimaric acid, but it is not the optical antipode of *l*-pimaric acid and even the carbon-skeleton is different from that of the members of the abietic acid series (see below). Unlike abietic acid, *d*-pimaric acid (m.p. 211°, $[\alpha]_D + 75^\circ$) is stable to heat and to mineral acids. The unsaturated acid forms a crystalline hydrochloride from which it can be regenerated by treatment with quinoline.¹⁶ The preparation of *d*-pimaric acid from fresh galipot involves no isomerization but only extraction

¹⁴ Palkin and Harris, *J. Am. Chem. Soc.*, **56**, 1935 (1934).

¹⁵ Ruzicka and Balaz, *Ann.*, **460**, 202 (1928).

with ether and crystallization of the acid or of its sodium salt, the yield being about 2%.¹⁷

The Relationship of Abietic Acid to Retene. Chemical investigations of the acids of rosin were undertaken in the early part of the nineteenth century. The name "abietic" acid was first applied by Baup¹⁸ in 1826 to a product obtained from *Pinus abies*. Much of the early work had to do with the isolation of acids from different sources, and many substances of doubtful individuality were described and named in the course of this work. It gradually became recognized that the acids most suitable for the investigation of structure are *d*-pimaric acid and abietic acid which, in contrast to many primary resin acids, are stable, or comparatively stable, substances. Attention was directed particularly to the more abundant abietic acid. The occurrence of the acid in conjunction with terpenes suggested a relationship to these substances, but a still more enlightening relationship was that established between abietic acid and the aromatic hydrocarbon retene, $C_{18}H_{16}$.

A hydrocarbon preparation which apparently was essentially retene in an impure condition was described as early as 1837 by Trommsdorff,¹⁹ who studied a substance found by Fikentscher in fossilized pine wood from a peat bed, along with fichtelite (see below). The hydrocarbon was obtained in a purer form in 1858 by Knauss from the pine tar oil resulting from the destructive distillation of pine wood or of rosin. This material was studied and described by Fehling²⁰ and by Fritzsche.²¹ Pure retene (Gr. *rhétine*, pine tree) is a colorless, crystalline substance melting at 98-99°. Only small amounts of retene were obtained from the technical oils produced industrially by the high temperature distillation of resins and oleoresins, but in 1887 patents were assigned for a method of increasing the yield of retene by heating the oils with sulfur until no further hydrogen sulfide is evolved, followed by distillation of the residue.²² The pine tar oils probably contain various products of the pyrolysis of abietic acid. In 1903 Vesterberg²³ submitted pure abietic acid ($C_{19}H_{20}COOH$) to reaction with sulfur and obtained retene ($C_{18}H_{16}$) as the chief product. This is the first instance of the dehydrogenation of a hydroaromatic compound to a completely aromatic type. The method of dehydrogenation introduced by Vesterberg has been of the greatest importance in the investigation of other natural products, as will be detailed in later chap-

¹⁷ Rusicka and Balas, *Helv. Chim. Acta*, 6, 677 (1923); Rusicka, de Graaff, Goldberg and Frank, *ibid.*, 15, 915 (1932).

¹⁸ Baup, *Ann. chim. phys.*, [2], 31, 108 (1826).

¹⁹ Trommsdorff, *Ann.*, 21, 126 (1837).

²⁰ Fehling, *ibid.*, 106, 398 (1858).

²¹ Fritzsche, *J. prakt. Chem.*, 75, 281 (1858); 82, 321 (1861).

²² See for example, *Ber.*, 21E, 553 (1888).

²³ Vesterberg, *ibid.*, 36, 4200 (1903).

ters, and in the case of abietic acid Vesterberg's observation provided a most valuable clue to the structure of the substance. Retene contains all but two of the original carbon atoms of the resin acid and the structure of the hydrocarbon was in large part already known at the time when it became a matter of importance in resin acid chemistry.

The Structure of Retene. Comprehensive studies of retene and its derivatives were carried out by Wahlforss²⁴ in Finland in 1869 and by Ekstrand²⁵ in Sweden in 1877, but neither investigator was able to arrive at a very clear conception of the structure. Bamberger²⁶ started work on the problem in the Munich laboratory in 1884 and among other observations he characterized an orange oxidation product, first prepared by Wahlforss, as an ortho quinone closely resembling phenanthrenequinone. The colored retenequinone could be reduced to a colorless hydroquinone, it yielded a quinoxaline derivative, and it could be oxidized to a dicarboxylic acid resembling diphenic acid. The quinone presented a perplexing problem, however, for although it could be obtained from retene under the conditions employed for the preparation of phenanthrenequinone, and although retene could be regenerated on zinc dust distillation, the analytical figures available did not indicate that the quinone and the hydrocarbon are related to one another in the normal manner. There were other apparent contradictions in the early work and although a number of derivatives and degradation products of retene had been prepared in a pure condition the relationship between them remained obscure until special attention was given to the problem of analysis in the investigations of Bamberger and Hooker²⁷. It was discovered that many of the compounds of the retene group burn with great difficulty and that macrocombustions conducted in the ordinary way with a copper oxide filling often lead to erroneous results. By using a very small sample, mixing it with lead chromate, and conducting the combustion in a tube packed with lead chromate, Bamberger and Hooker were able to obtain accurate analytical results. Several of the empirical formulas previously assigned were found to require revision, and the new formulas made it possible to interpret the older observations and to extend the work on a rational basis. In a very short time Bamberger and Hooker were able to untangle the accumulated observations in the field and to carry the determination of structure nearly to completion.

In the most important series of degradations it was established conclusively that retene is an alkyl derivative of phenanthrene and that the

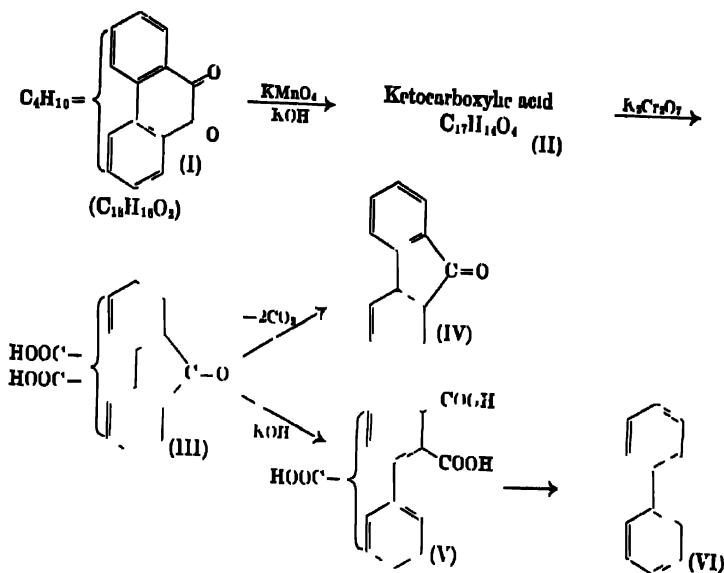
²⁴ Wahlforss, *Jahresber. Chem.*, 501 (1869).

²⁵ Ekstrand, *Ann.*, 185, 75 (1877).

²⁶ Bamberger, *Ber.*, 17, 453 (1884), 18, 81 (1885).

²⁷ Bamberger and Hooker, *ibid.*, 18, 1024, 1030, 1750 (1885), *Ann.*, 229, 102 (1885).

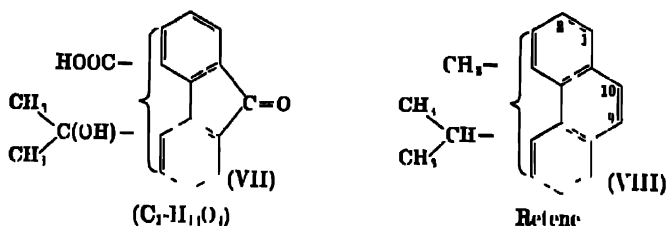
central ring is the one involved in the formation of the quinone. The oxidation of retenequinone (I) with alkaline permanganate proceeds by way of a benzilic acid rearrangement and results in the elimination of one carbonyl group from the quinonoid ring (see page 15). The character of the oxidation product II will be discussed below. The



product of further oxidation, III, was shown to be a fluorenone dicarboxylic acid by its conversion to fluorenone (IV) and diphenyl (VI). The presence of two carboxyl groups in the fluorenone derivative III shows that the $C_{17}H_{10}$ -residue of the quinone (I) must be distributed between two alkyl groups. The location of one of these groups can be inferred from the fact that the tricarboxylic acid V easily forms an anhydride whereas the dibasic acid III does not do so. One carboxyl group must be in the ortho position to the carbonyl group of III and to the carboxyl group which is liberated in the fusion with alkali. It follows that an alkyl group of the original quinone is situated at C_1 , adjacent to a quinonoid carbonyl group.

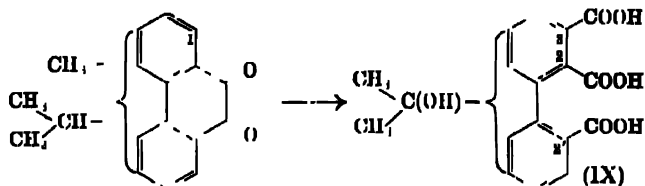
According to this evidence retenequinone is either a diethyl- or a methylpropyl-phenanthrenequinone, and the character of the keto acid (II, above) obtained as the first oxidation product distinguishes between the two possibilities. Since the carbon atom lost in the formation of this keto acid is known to come from the quinonoid nucleus, the four carbon atoms of the original alkyl groups are still intact. The carboxyl group

of the oxidation product (II) could not have come from an ethyl or propyl group without the loss of carbon and it must have arisen from a methyl group. The second alkyl radical therefore is a propyl group, and a choice between the normal and the branched chain structure can be made from the fact that the keto acid II contains one oxygen atom not accounted for in the carbonyl and carboxyl groups. Since the *n*-propyl group is not susceptible to partial oxidation, while an isopropyl group can be hydroxylated by alkaline permanganate, the degradation product may be assigned the structure VII. Retene is a methylisopropylphenan-



threne, in which one of the alkyl groups is located at C₁ and the other is at some position other than C₂, C₃, or C₁₀.

This much of the structure of retene was established by Bamberger and Hooker in 1885. The problem of locating completely the alkyl groups remained unsolved for twenty-five years, although isolated observations²⁸ indicated that the methyl and the isopropyl groups probably are situated at C₁ and C₇ or at C₇ and C₁. Finally in 1910 Bucher²⁹ at Brown University achieved a complete proof of structure in a series of brilliantly executed oxidations. By the action of potassium permanganate on retenequinone in pyridine solution, Bucher obtained a tribasic hydroxy acid (IX) in which the carboxyl groups at C₁ and C₇ obviously come from the opening of the quinonoid ring. While diphenic acid itself



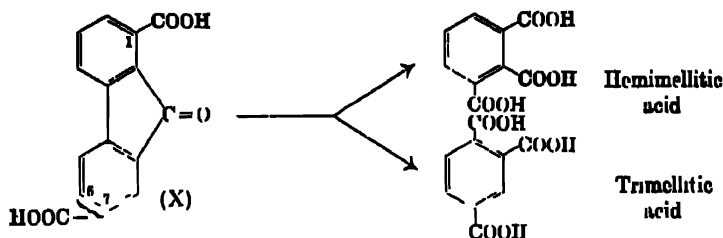
sublimes unchanged, although it can be converted into an anhydride by the action of acetyl chloride, the degradation product (IX) forms an anhydride on being heated at the melting point. The third carboxyl

²⁸ Fortner, *Monatsh*, 25, 443 (1904); Lux, *Ind*, 29, 763 (1908); Schultze, *Ann*, 359, 120 (1908)

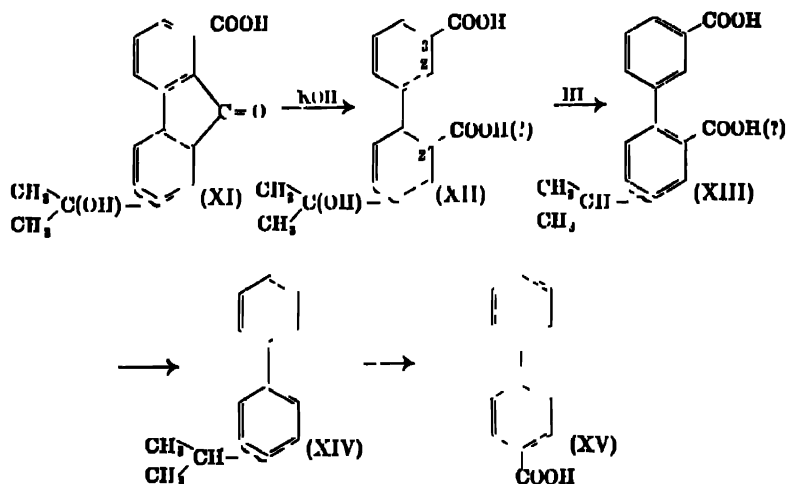
²⁹ Bucher, *J. Am. Chem. Soc.*, 32, 374 (1910).

group therefore is ortho to one of the other groups and may be placed at C_8 , corresponding to position C_1 in retene. The alkyl group originally occupying this position could not be the isopropyl group, for the C_8 -residue is still retained in the product in a hydroxylated condition. The carboxyl group appearing at C_8 in the oxidation product comes from a methyl group at C_1 in retene and retenequinone.

Some indication of the position of the isopropyl group was furnished by Bucher's observation that the fluorenone dicarboxylic acid (X) of Bamberger and Hooker yields hemimellitic acid and trimellitic acid on oxidation with potassium permanganate, showing that the unlocated



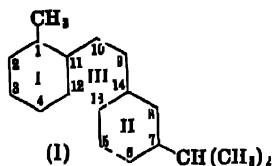
carboxyl group in the dibasic acid (X) is in the ring not occupied by the first group and at either C_6 or C_7 . A final decision was obtained by the degradation of Bamberger and Hooker's hydroxyfluorenone carboxylic



acid (XI). The position of the carboxyl group liberated on opening the fluorenone ring of XI by alkali fusion is not known. The more probable formula is that (XII) in which the new group is as far removed as possible from the group already present. Since both of the groups were

Such a substituent, whether it is an alkyl, carboxyl, or hydroxyl group, will be referred to as a tertiarily bound, or merely a "tertiary," group, for it is situated on a (quaternary) carbon atom which is joined to three other carbon residues.

Abietic acid appears from this evidence to be 1-methyl-7-isopropyl-perhydrophenanthrene (I) with a methyl group at any of the positions



C_{11} , C_{12} , C_{13} , or C_{14} , and with an unlocated carboxyl group. In the formula the nuclear hydrogen atoms are omitted for convenience and the three rings are numbered in the same sequence as the carbon atoms. Since the number of carbon atoms in the resin acid is an even multiple of the C_5 -isoprene unit, and since the acid occurs along with various terpenes, all of which are known to be constructed of condensed isoprene groups, it was early suspected that abietic acid is a diterpene acid, and the hypothesis that the carbon skeleton is resolvable into four isoprene residues served as a useful guide in considering the most probable formulations. More evidence was required, however, to limit the number of possibilities in terms of the "isoprene rule."

The composition of abietic acid is such as to indicate the presence of either two double bonds or two additional rings. The acid is unsaturated³² to permanganate and to halogens, and extensive studies of various addition reactions have indicated clearly the presence of two ethylenic linkages. The addition of two molecules of hydrogen bromide was accomplished by Levy,³³ who also succeeded in obtaining a tetrahydroxyabietic acid by oxidation with permanganate under controlled conditions.³⁴ Ruzicka and Meyer³⁵ isolated a dihydroxy acid under similar conditions. In the presence of platinum catalyst, abietic acid readily absorbs one mole of hydrogen, and a tetrahydro derivative is formed more slowly.⁹ The molecular refraction of the esters of abietic acid⁹ and the behavior of the acid toward perbenzoic acid³⁶ also point to the presence of two double bonds. The action of ozone is anomalous, for abietic acid forms a triozonide, perhaps as the result of a dehydro-

³² It gives the Liebermann-Burchard test (page 117) characteristic of unsaturated sterols, La Lande, *J. Am. Chem. Soc.*, **55**, 1516 (1933).

³³ Levy, *Ber.*, **40**, 3659 (1907); *Z. anorg. Chem.*, **81**, 145 (1913).

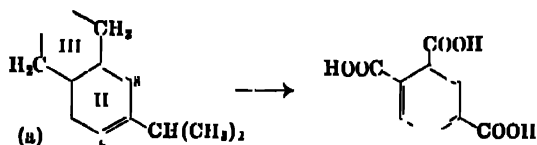
³⁴ Levy, *Ber.*, **42**, 4305 (1909), **59**, 1802 (1926); **61**, 616 (1928).

³⁵ Ruzicka and J. Meyer, *Helv. Chim. Acta*, **6**, 1097 (1923).

³⁶ Ruzicka, Huyser and Seidel, *Rec. trav. chim.*, **47**, 363 (1928).

generating action of the reagent or through the formation of a molecular compound.⁹

Evidence that the two double bonds are conjugated is furnished by the observation³⁷ that abietic acid esters form diene addition products with maleic anhydride. Although the determination of the location of the unsaturated centers in a complicated ring system is a particularly difficult matter, especially in a case where the bonds appear to be rather mobile, there are two pieces of evidence indicating that the conjugated system is associated with the ring (II) carrying the isopropyl group. Oxidation with nitric acid³⁸ gives the aromatic trimellitic acid as one degradation product. The 1,2,4-acid cannot come from ring I or ring III, and the fragment must arise from the ring (II) carrying an alkyl group in a β -position. If this ring has a dihydrobenzenoid structure, as in the partial formula (a), the aromatization under the influence of nitric acid is understandable. Independent evidence that the isopropyl group is attached to



an unsaturated carbon is furnished by the isolation of isobutyric acid as one of the oxidation products of abietic acid,^{39, 40} indicating the presence of a double bond at either C_6-C_7 or C_7-C_8 : $(CH_3)_2CHC=CH \longrightarrow (CH_3)_2CHCOOH$. The above disposition of the diene system is regarded as providing the best account in stereochemical terms of the Diels-Alder reaction with maleic anhydride.

On energetic oxidation of abietic acid with potassium permanganate the unsaturated ring is destroyed along with the central nucleus. Ruzicka^{40 41} isolated from the reaction mixtures a C_{12} -acid and a C_{11} -acid which are identified as containing the original ring I by the presence of the nuclear methyl group characteristic of both abietic acid and retene. The yields are very poor, only 22-24 g. of each acid being obtained from 3 kg. of starting material, but the acids have been isolated in both permanganate and nitric acid⁴² oxidations. It will be shown presently that the acids can be represented by the formulas (b) and (c), the former probably being the precursor of the latter. The separation of the acids is accomplished

³⁷ Ruzicka, Ankersmit and Frank, *Helv. Chim. Acta* 15, 1289 (1932); Arbusow, *J. Gen. Chem., U.S.S.R.*, 2, 806 (1932).

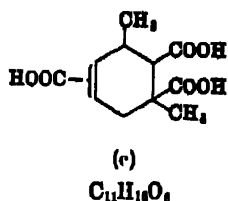
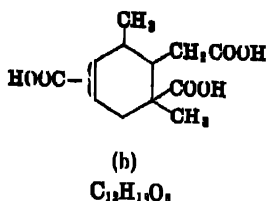
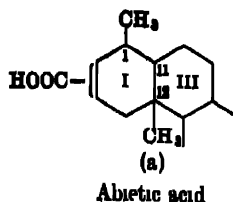
³⁸ Ruzicka and Pfeiffer, *Helv. Chim. Acta*, 8, 632 (1925).

³⁹ Levy, *Ber.*, 42, 4303 (1909).

⁴⁰ Ruzicka, J. Meyer and Pfeiffer, *Helv. Chim. Acta*, 8, 637 (1925).

⁴¹ Ruzicka, Goldberg, Huyser and Seidel, *ibid.*, 14, 545 (1931).

⁴² Levy, *Ber.*, 62, 2494 (1929).



either by partial esterification, or by partial hydrolysis of the ester mixture, the C_{12} -acid reacting more rapidly or completely in each case. Two of the carboxyl groups represent remnants of ring III, while the third must correspond to the original carboxyl group of abietic acid. Of the two methyl groups, one is that originally located at C_1 and the other is the tertiarily bound group eliminated in the conversion of abietic acid into retene. The latter group must be at C_{11} , or at C_{12} , and Ruzicka⁴¹ was able to distinguish between the two possibilities by the dehydrogenation (and decarboxylation) of the C_{12} - and C_{11} -acids with selenium. The first acid gave 1,2,3-trimethylbenzene, the second yielded *m*-xylene. The tertiary methyl group and the nuclear methyl group at C_1 therefore bear the 1:3 relationship to one another and the only possible location for the former group is at C_{12} . In confirmation of this point Ruzicka and Waldmann⁴¹ isolated 1,3-dimethylcyclohexanone-2 in small yield in another oxidation.

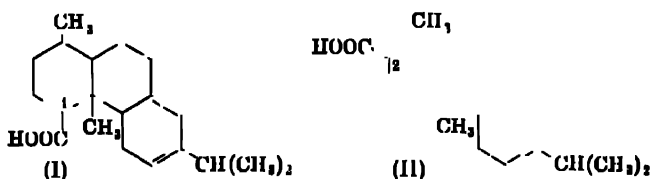
The most perplexing point in the determination of the complete structure was the problem of locating the carboxyl group of the resin acid, beyond the observation that it is situated in ring I. To early workers it seemed quite significant that abietic acid is esterified only with difficulty. Prolonged boiling with alcohol containing about 20% of sulfuric acid is necessary for complete conversion to the ethyl ester,⁴² and the other esters are more conveniently prepared by methods which are not subject to steric hindrance, for example, by the action of alkyl sulfates or alkyl *p*-toluene sulfonates on the silver or sodium salt of the acid. It was suggested as early as 1901 that the carboxyl group probably is tertiarily bound, as for example at C_1 or C_{11} in the above partial formula.⁴⁴ This idea was temporarily abandoned, however, as the result of an observation made by Ruzicka at an early stage in his important work on the problem (1922). It occurred to Ruzicka that the elimination of the carboxyl group in the conversion of abietic acid into retene might not be merely a normal decarboxylation at the high temperature of reaction but rather the result of a special situation of the carboxyl group, such as a

⁴¹ Ruzicka, Schinz and J. Meyer, *Helv. Chim. Acta*, 6, 1077 (1923)

⁴⁴ Fahrion, *Z. anorg. Chem.*, 14, 1197 (1901)

tertiary location. The observation ⁴⁵ that abietic acid methyl ester likewise yields retene on reaction with sulfur seemed to support the latter view, the loss of the ester group being out of harmony with the idea of a decarboxylation of the ordinary type. At the time, however, so little was known concerning the dehydrogenation of hydroaromatic acids and esters that it seemed possible that sulfur regularly eliminates oxygen-containing groups regardless of their location. Consequently Ruzicka and Meyer ⁴⁵ undertook to reduce the carboxyl group completely and to determine if the corresponding alkyl group is eliminated on dehydrogenation. Ethyl abietate (RCOOC_2H_5) was reduced by the Bouveault method (sodium and alcohol) to abietinol (RCH_2OH), and the primary alcohol was dehydrated by treatment with phosphorus pentachloride, giving the triply unsaturated hydrocarbon "methylabietin" ($\text{C}_{20}\text{H}_{10}$). On reaction with sulfur this yielded not retene but its homologue, "methylretene," $\text{C}_{18}\text{H}_{10}$. It was assumed that the extra methyl group which survives the aromatization is joined to the phenanthrene nucleus, and that the original carboxyl group occupies a corresponding position in one of the six-membered rings. This work was taken as a proof that the carboxyl group is not present in tertiary linkage.

By 1932, after the accumulation of the greater part of the other evidence cited above, it appeared that formulas I and II alone are consistent with all of the facts and at the same time in conformity with the isoprene rule. In that year, however, it was discovered independently in three dif-



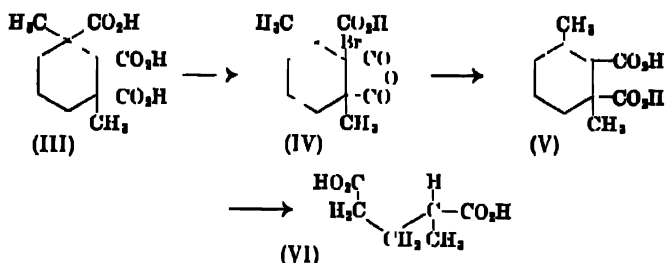
ferent laboratories that the previous conclusions were not all valid and that abietic acid does not have the carbon framework of either I or II. Vocke ⁴⁶ at Munich was the first to suggest a revision, but his results were not entirely conclusive. An attempted oxidative degradation of the diphenyl carbinol obtained from the methyl ester of tetrahydroabietic acid and phenyl magnesium bromide was unsuccessful, the oxygen-containing group being so resistant to attack as to suggest a tertiary attachment. Since, according to Bistrzycki, ⁴⁷ secondarily bound carboxyl groups are rather stable to concentrated sulfuric acid while acids with tertiary

⁴⁵ Ruzicka and J. Meyer, *Helv Chim Acta*, 5, 581 (1922)

⁴⁶ Vocke, *Ann*, 497, 247 (1932).

⁴⁷ Bistrzycki and v. Siemiradski, *Ber*, 39, 51 (1906), 41, 1665 (1908), Bistrzycki, Reintke and Mauron, *ibid*, 38, 534 (1905), 40, 4370 (1907)

groups often lose carbon monoxide on warming, Vocke applied this diagnostic test to tetrahydroabietic acid and to Ruzicka's tribasic C_{11} -acid. The results were inconclusive, but again rather suggestive of the presence of a tertiarily bound carboxyl group, for these acids lose some carbon monoxide on being heated with sulfuric acid. Vocke then carried out a degradation of the C_{11} -acid and concluded that the results were best interpreted on the basis of formula III for this oxidation product.⁴⁸ Energetic treatment with bromine and red phosphorus gave an α -bromoanhydride-



acid (IV) together with the corresponding acid bromide. The $-COBr$ group of the latter compound is highly resistant to hydrolysis, which argues for its attachment to a tertiary carbon atom. The elimination of hydrogen bromide from IV by means of alkali was accompanied by the loss of carbon dioxide, giving a dibasic acid of properties consistent with formula V. The product of ozonization was not isolated but it gave a positive iodoform reaction, indicating an original $CH_3C=$ group. Further oxidation of the ozonide gave methylglutaric acid (VI). As expected for a β,γ -unsaturated acid, V was converted by dilute sulfuric acid into a stable lactone.

Vocke recognized that, although the degradation supported the formulation III for Ruzicka's acid, a possible, if not very satisfactory, explanation of the transformations can be given on the basis of the alternate formulas VII and VIII for the C_{11} -acid and the dibasic acid obtained from it. It was only with considerable reservation that Vocke suggested



that the carboxyl group of abietic acid probably is situated at C_{11} , along with the nuclear methyl group.

⁴⁸ Arbuzov and Schapachinskaja, *Ber.*, **68**, 437 (1935), attempted the synthesis of an acid of this structure but they did not obtain a crystalline product, possibly because of the presence of several stereoisomerides.

Shortly after the appearance of Voecke's paper, Ruzicka⁴⁹ reported the results of an investigation of the problem from an entirely different point of view. Ruzicka undertook to distinguish between what he had regarded as the two most probable formulas for abietic acid (I and II above) by the degradation of "methylretene." The extra methyl group might be identified as a carboxyl group in a suitable oxidation product. In his work on *d*-pimaric acid (see below) Ruzicka had found that alkyl phenanthrenes can be oxidized to phenanthrene carboxylic acids by means of alkaline potassium ferricyanide,⁵⁰ a reaction which had been employed by Weissgerber and Kruber⁵¹ in the naphthalene series. Retene yields phenanthrene-1,7-dicarboxylic acid, along with diphenyl-2,3,2',4'-tetracarboxylic acid, a product of further oxidation. From "methylretene," Ruzicka, de Graaff and Muller⁴⁹ obtained, instead of the expected tribasic acid, a dibasic acid identical with the 1,7-derivative from retene. The additional methyl group is not in the phenanthrene nucleus! The only position possible is in a side chain, and indeed the extra carbon atom must be combined with the original methyl group at C₁ in the form of an ethyl group. The supposed "methylretene" is in fact 1-ethyl-7-isopropylphenanthrene.

This conclusion was reached independently and at practically the same time by R. D. Haworth⁵² at the University of Durham. With the object of identifying the hydrocarbons resulting from the dehydrogenation of the resin acids and their derivatives, Haworth had perfected methods for the synthesis of a wide variety of alkyl phenanthrenes. This work will be described in a special section, and it is sufficient at present to state that after synthesizing the 4-methyl derivative of retene and finding that it is not identical with "methylretene," Haworth was led to suspect that the latter hydrocarbon contains an ethyl group. In the work cited, Haworth established the identity of the hydrocarbon by comparison with a sample of 1-ethyl-7-isopropylphenanthrene obtained by synthesis.

The facts could be explained by assuming the presence of the grouping $-\text{CH}_2\text{COOH}$ at position C₁ in abietic acid, but a primary linkage of the carboxyl group is wholly inconsistent with the hindrance evident in the esterification reaction. A more probable interpretation, suggested by both Ruzicka and Haworth, is that the carboxyl group is located at

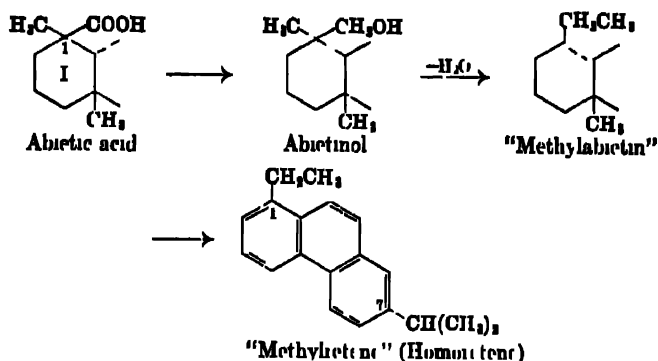
⁴⁹ Ruzicka, de Graaff and H. J. Muller, *Helv. Chim. Acta*, **15**, 1300 (1932).

⁵⁰ Ruzicka, de Graaff and Hosking, *ibid.*, **14**, 233 (1931).

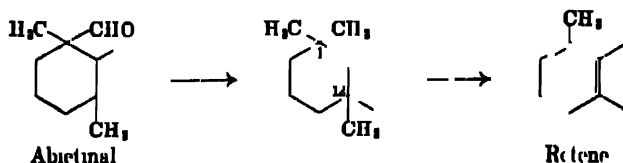
⁵¹ Weissgerber and Kruber, *Ber.*, **52**, 352 (1919).

⁵² R. D. Haworth, *J. Chem. Soc.*, 2717 (1932).

C₁₁, along with the methyl group, and that a rearrangement of the Wagner-Meerwein type occurs in the dehydration of abietinol:



If the structures are as pictured, abietinol is an alcohol of a type susceptible to dehydration only by virtue of a molecular rearrangement, for it contains a tertiary bound primary alcoholic group. This view of the reaction series was later substantiated by the results of a degradation which involved no dehydration. Ruzicka and co-workers⁵³ converted abietinol into the corresponding aldehyde and reduced the carbonyl group of this substance by the Wolff-Kishner method (heating the semicarbazone with sodium ethylate). The resulting hydrocarbon gave retene, rather than its homologue, on dehydrogenation:

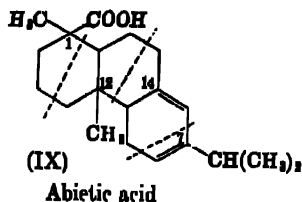


The new methyl group at C₁₁, like that at C₁₀, is eliminated in the course of the aromatization of the ring in question because it is tertiary bound. It may be inferred that the same factor is responsible for the ready elimination of the original carboxyl or carboethoxyl group in the dehydrogenation of abietic acid or its ester.⁵⁴

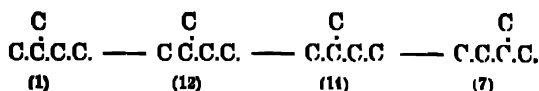
On the basis of the new evidence abietic acid has been assigned the structure IX, which is uncertain only with regard to the location of the double bonds. It is interesting that the formula is resolvable into four

⁵³ Ruzicka, Waldmann, Meier and Hölzl, *Helv. Chim. Acta*, 16, 109 (1933).

⁵⁴ From the work of Darzens [Compt. rend., 183, 748 (1926), and later papers] it appears that a secondary bound carboxyl group similar to that indicated in Ruzicka's early formulas for abietic acid normally withstands dehydrogenation with sulfur, for example in the case of 4-methyl-1,2,3,4-tetrahydronaphthalene-2-carboxylic acid. That 4-methyl-1,2,3,4-tetrahydronaphthalene-1-carboxylic acid loses carbon dioxide on dehydrogenation with sulfur or selenium [Darzens and Lévy, *ibid.*, 109, 1181 (1904)] can be attributed to the influence of the unsaturated aromatic ring adjacent to the carboxyl group.



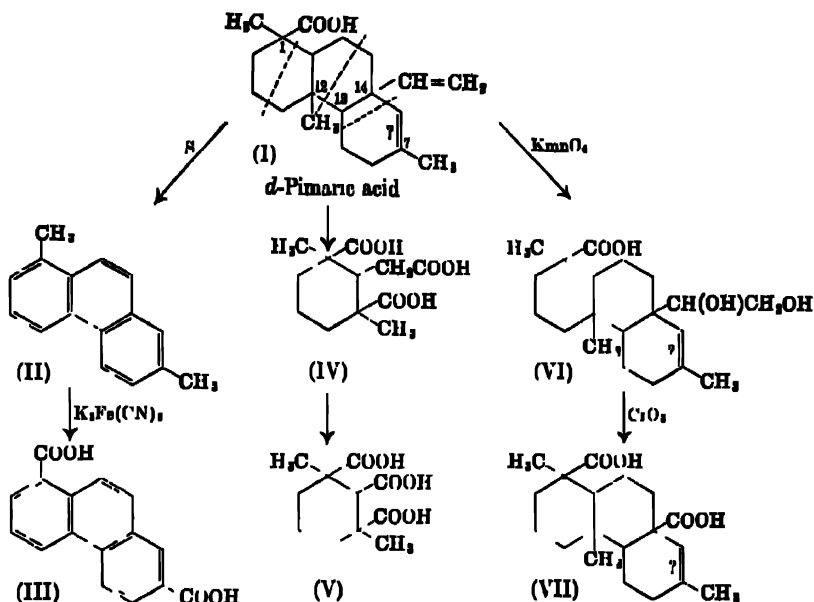
isoprene units, as indicated by the dotted lines, although the isoprene rule actually was of little value in arriving at the true structure. The isoprene groups are arranged irregularly, the chain being as follows:



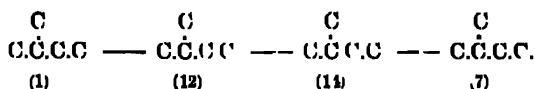
d-Pimaric Acid. While investigating the chemistry of abietic acid, Ruzicka and his co-workers made parallel studies of the acid-stable isomer, *d*-pimaric acid.³⁵ Degradations similar to those described above led to the establishment of a structural formula which is highly probable, if not entirely certain, and which is incomplete only with respect to the location of a nuclear double bond. The chief difference between *d*-pimaric acid and abietic acid, according to Ruzicka's formula (I), is that instead of the isopropyl group at C₇ there is a methyl group at C₇ and a vinyl group at C₁₄.

Dehydrogenation gives the hydrocarbon pimarane (II), which was identified as 1,7-dimethylphenanthrene by oxidation to III (Ruzicka) and by synthesis (Haworth). The reduction of the ester of *d*-pimaric acid by the Bouveault method, followed by dehydration of the primary alcohol and dehydrogenation of the resulting hydrocarbon, gives 1-ethyl-7-methylphenanthrene. This was at first regarded as a "methylpimarane," but it was found to yield III on oxidation and the structure was established by synthesis. The formation of the same C₁₃- and C₁₁-acids (IV and V) from *d*-pimaric acid as from abietic acid establishes the structure of ring I. The production of formaldehyde as an ozonization product of *d*-pimaric acid is evidence of the presence of the group =CH₂, and the conversion of the resin acid through the dihydroxy acid VI to the dibasic nor-acid (having one less carbon atom) VII shows that a vinyl group is present. Since the vinyl group is lost in the course of the dehydrogenation to pimarane, and since it is not associated with ring I, the only positions available are C₁₃ and C₁₄. There is no evidence on this

³⁵ Ruzicka and co-workers, *Helv. Chim. Acta*, 6, 877 (1923); 7, 875 (1924); 14, 233 (1931); 15, 915, 1289 (1932); *Ann.*, 460, 302 (1928); *Rec. trav. chim.*, 47, 303 (1928).

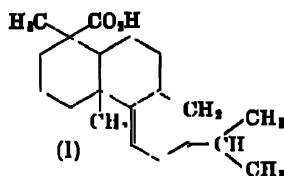


point, but a choice is possible on the basis of the isoprene rule. The formula above, in which the vinyl group is placed at C_{14} , is divisible into four isoprene units while this is not true with the vinyl group at C_{13} . The arrangement in formula I is regular:



The two double bonds differ considerably in reactivity, and from the formation of the dihydroxy acid VI and its conversion to VII it may be inferred that the double linkage of the vinyl group is more reactive than the unlocated nuclear double bond. Hydrogenation in the presence of platinum black proceeds only to the dihydro stage even in warm glacial acetic acid solution, while a mixture of tetrahydro compounds can be obtained with the use of Adams' platinum oxide catalyst. With perbenzoic acid the first atom of oxygen is taken up rapidly and the second one slowly. The nuclear double bond appears to be inaccessible to certain reagents, for *d*-pimaric acid adds only one mole of bromine or of hydrogen chloride, and it forms only a mononitrosite. There are indications that the two double bonds are not conjugated, and no addition product is formed with maleic anhydride.

The Primary Constituents of Oleoresins. Continuing an investigation started by Voeko,⁵⁶ Kraft⁵⁷ found that the crystalline acids obtained by allowing fresh resin from American slashpines and longleaf pines to stand, in the absence of air, consist of mixed crystals of *d*-pimaric acid with varying amounts of *l*-pimaric acid, depending upon the extent to which it has become isomerized to abietic acid. Kraft made the significant observation that the ultraviolet absorption spectrum of *l*-pimaric acid is suggestive of a compound having three conjugated double bonds, for example, such as I. The absorption maximum is at 272.5 mμ, as compared with 237.5 mμ for abietic acid (two conjugated double bonds). Abietic acid probably does not occur as such in nature, and it may owe its origin to the ready cyclization of primary substances of the type of I under the influence of heat or of acids.



Kraft's conclusion as to the nature of the so-called sapinic acids, or primary constituents of the oleoresins of conifers, was confirmed in independent work by Hasselstrom and Bogert.⁵⁸ Several sapinic acids melting in the neighborhood of 140-150° and differing considerably in the degree of levorotation were shown to contain *d*-pimaric acid by hydrogenation to its dihydro derivative, which was easily isolated by virtue of its sparing solubility in methanol. The presence of *l*-pimaric acid was established by isomerization to abietic acid with hot glacial acetic acid. Recognizing the awkwardness of the designation "*l*-pimaric acid" for the labile substance, which is not related to *d*-pimaric acid, Hasselstrom and Bogert suggest that the name be changed to "*l*-sapietic acid." Since the acid is related to abietic acid and yields retene rather than pimaranthrene when heated with sulfur, this modification of the earlier nomenclature appears highly appropriate.

Agathic Acid. Agathic acid (or "agathic dicarboxylic acid"), from Manila or kauri copal, is not a hydrophenanthrene derivative but it is isomerized by formic acid to an iso-acid having this ring system. From such observations as have been made, and in consideration of the isoprene rule, Ruzicka and Hosking⁵⁹ have suggested as a working hypothesis the

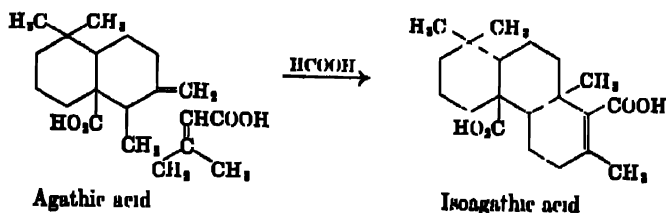
⁵⁶ Voeko, *Ann.*, 506, 11 (1934).

⁵⁷ Kraft, *ibid.*, 520, 133 (1935).

⁵⁸ Hasselstrom and Bogert, *J. Am. Chem. Soc.*, 57, 2118 (1935).

⁵⁹ Ruzicka and Hosking, *Ann.*, 469, 147 (1929); *Helv. Chim. Acta*, 13, 1403 (1930); 14, 203 (1931).

structures indicated in the accompanying formulas. On dehydrogenation with sulfur or selenium isoagathic acid yields pimanthrene, while



agathic acid gives a mixture of 1,2,5-trimethylnaphthalene and pimanthrene. The naphthalene derivative probably is the normal product, for pimanthrene may well arise as the result of a partial isomerization. One of the carboxyl groups of isoagathic acid is easily lost on heating, with the formation of isonoragathic acid, and this group therefore is considered to be located on an unsaturated carbon atom. The labile group of isoagathic acid, like the second acid group, is subject to steric hindrance, as shown in hydrolysis experiments with the diester. This is true of only one of the carboxyl groups of agathic acid, the diester being easily hydrolyzed to a monoester. It is inferred that the carboxyl group which occupies a hindered position only after the isomerization is joined to a carbon atom involved in the formation of the new ring.

Fichtelite. Certain fossil resins contain, along with varying proportions of retene, a completely saturated hydrocarbon resembling paraffin in both chemical and physical properties and known as fichtelite (Ger. *Fichte*, pine). The hydrocarbon, m. p. 46.5°, was first isolated by Broms⁸⁰ from material obtained by Fickelscher from remnants of pine trunks in a peat bed of the Fichtelgebirge region of Bavaria. From the same material Trommsdorff previously had obtained retene, probably admixed with some fichtelite. The material has the appearance of dried pine wood and the hydrocarbons are present in a crystalline condition and are found mostly between the annual rings of the fossilized wood. Fichtelite and retene have been found in other peat beds and lignite beds from pine forests and the substances undoubtedly come from the resin acids originally present in the live trees.

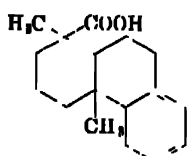
The characterization of fichtelite is not a simple matter because the substance is very resistant to attack by chemical agents. On the basis of analyses, and its occurrence with retene, the hydrocarbon was regarded by early workers as a perhydro derivative of retene of the formula

⁸⁰ Broms, *Ann.*, 37, 304 (1841).

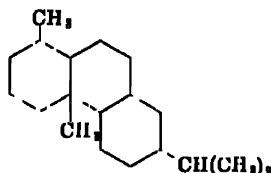
$C_{18}H_{18}$.⁶¹ A definite relationship was established by the observation that the hydrocarbon yields retene on dehydrogenation with sulfur.⁶² Recognizing that ordinary analyses do not distinguish between the formulas $C_{18}H_{18}$ and $C_{18}H_{24}$, Ruzicka and Waldmann⁶³ undertook to settle this point by following quantitatively the dehydrogenation of fichtelite with palladium charcoal at 330-370°. If the hydrocarbon has the same number of carbon atoms as retene, the gas should consist solely of hydrogen. Analysis of the gas, which was obtained in 90% yield, indicated the presence of methane, and the ratio corresponded approximately with that required by the equation:



This indicates the presence of an additional methyl group in tertiary linkage. Since fichtelite probably arises from abietic acid in the process of decay, Ruzicka and Waldmann have suggested that it has a corresponding structure.



Abietic acid



Fichtelite

THE SYNTHESIS OF ALKYL PHENANTHRENES

The development of synthetical methods suitable for the preparation of methyl derivatives of retene and pimanthrene was undertaken independently in 1932 by R. D. Haworth in England and by Bardhan and Sengupta in India with the object of identifying the supposed "methyl-retene" and "methylpimanthrene." As stated above, this objective was achieved. The work is of further significance because the new methods later proved to be of the greatest value in the synthesis of compounds related to natural products other than the resin acids. The subsequent history differed from that of the Pschorr synthesis (page 28), which served the purpose for which it was designed but found few other applications. The Pschorr method was not suitable for the purpose under discussion for the reason that the types of intermediates required are not available.

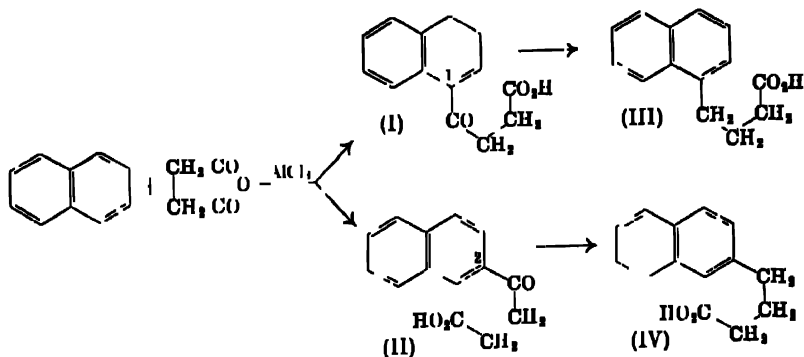
⁶¹ Heli, *Ber.*, 22, 498 (1889); Liebermann and Spiegel, *ibid.*, 22, 770 (1889); Spiegel, *ibid.*, 22, 3369, (1889); Ramberger, *ibid.*, 22, 635 (1889); Ramberger and Stramer, *ibid.*, 22, 3361 (1889)

⁶² Ruzicka, Balas and Schinz, *Helv. Chim. Acta*, 6, 692 (1923).

⁶³ Ruzicka and Waldmann, *ibid.*, 18, 611 (1935).

Haworth's Synthesis. The general method used by Haworth and his co-workers⁶⁴ consists in the synthesis and cyclization of a γ -arylbutyric acid, followed by the aromatization of the new six-membered ring. This method of obtaining polynuclear types was by no means new, and it had been employed before in the phenanthrene series for the production of hydro derivatives⁶⁵ and for the synthesis of 4-methylphenanthrene.⁶⁶ The contribution of Haworth consisted in elaborating a known synthetical method and in adapting it to a specific purpose.

For the synthesis of a phenanthrene derivative, a γ -naphthylbutyric acid is required. The most convenient method of obtaining these acids is by the Friedel and Crafts reaction of succinic anhydride with a suitable naphthalene derivative, followed by the reduction of the resulting β -aroylpropionic acid. With naphthalene itself and in most other cases the reaction proceeds best in nitrobenzene solution at a low temperature.⁶⁷ Aluminum chloride dissolves in nitrobenzene and combines with the solvent to form a molecular compound⁶⁸ which is less reactive than the halide itself and less destructive of the sensitive naphthalene compounds. Mixtures of the 1- and 2-derivatives ordinarily are obtained, but a separation usually is possible. In the case of naphthalene itself the pure keto acids I and II can be prepared in yields as high as 26% and 47%, respectively.⁶⁹



The 2-acid invariably is the higher-melting and less soluble isomer and can be obtained pure by crystallization; the 1-acid is conveniently purified by the distillation of the methyl ester. In some cases the 2-acid forms a nicely crystalline sodium salt which affords an easy method of separa-

⁶⁴ R. D. Haworth and co-workers, *J. Chem. Soc.*, 1125, 1784, 2248, 2717, 2720 (1932); 454 (1934).

⁶⁵ Schroeter, *Ber.*, 37, 2003, 2025 (1904); Schroeter, H. Muller and Huang, *ibid.*, 62, 645 (1929).

⁶⁶ Radcliffe, Sherwood and Short, *J. Chem. Soc.*, 2293 (1931). See also the later synthesis of acenaphthene, Fieser and Peters, *J. Am. Chem. Soc.*, 54, 4375 (1932).

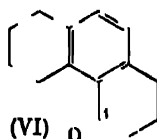
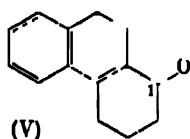
⁶⁷ German Patent 376,835 (1923); French Patent 636,065 (1928); Swiss Patent 131, 959 (1929), U. S. Patent 1,750,111 (1930).

⁶⁸ Kohler, *Am. Chem. J.*, 24, 385 (1900), 27, 241 (1902)

⁶⁹ Fieser and Peters, *J. Am. Chem. Soc.*, 54, 1347 (1932)

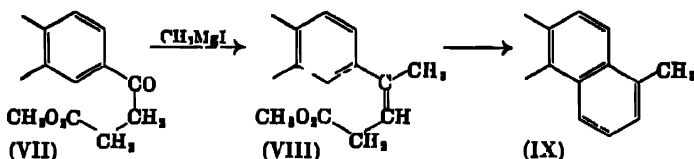
tion. β -Aroylpropionic acids also can be obtained from the reaction of succinic anhydride with one mole of an aryl Grignard reagent, but the yields are very poor.⁷⁰ For the reduction of the keto acids the Clemmensen method usually is satisfactory, although with a high-melting or sparingly soluble acid it often is necessary to add an organic solvent such as alcohol, acetic acid, or dioxane, or to use the lower-melting esters. In any case the results usually are considerably improved by providing a layer of toluene, for this keeps the carbonyl compound out of contact with the metal and inhibits dimolecular reduction.⁷¹

Haworth converted the acids III and IV into 1-keto-1,2,3,4-tetrahydrophenanthrene (V) and the isomeric 4-ketone (VI) by treatment



with 85% sulfuric acid. This convenient method is not always applicable, for in some cases much material is lost through sulfonation. Cyclization of this type often are effected in better yield by treating the acid chloride in a solvent with aluminum chloride or, in the case of compounds having a particularly reactive and sensitive aromatic nucleus, with the milder condensing agent stannic chloride. The ketones V and VI serve as starting materials for the synthesis of 1- and 4-substituted phenanthrenes. For example, with methyl magnesium iodide they are converted into carbinols which on distillation yield methyl dihydrophenanthrenes. These hydrocarbons on dehydrogenation afford 1- and 4-methylphenanthrene.

One variation in the synthesis consists in submitting the esters of keto acids such as I and II to reaction with one mole of Grignard reagent (inverse). In this way an alkyl group can be introduced in a position corresponding to that of the carbonyl group (formulas VII-IX). On

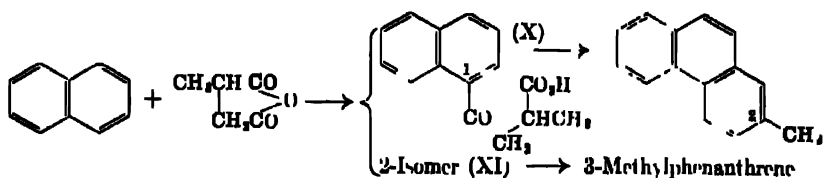


attempting to prepare 4-methylphenanthrene by this method Haworth found that the methyl group partially rearranged to the 1-position in the course of the dehydrogenation.

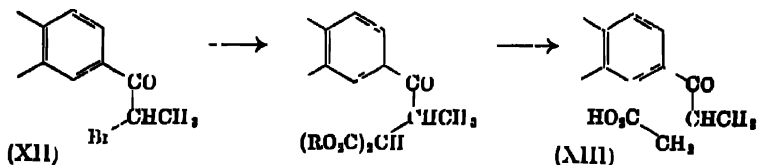
⁷⁰ Komppa and Rohrmann, *Ann.*, **509**, 250 (1934); Weissman, Blum-Bergmann and F. Bergmann, *J. Chem. Soc.*, 1370 (1935).

⁷¹ Observation of E. L. Martin

Further variation can be achieved by the use of methyl succinic anhydride in the Friedel and Crafts reaction. The naphthalene nucleus is substituted in both the α - and β -positions (X and XI), but the carbonyl

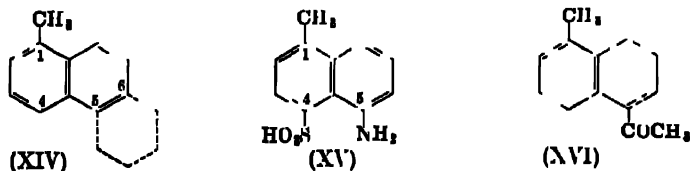


group of the anhydride which is furthest removed from the methyl group invariably is the one to become joined to the aromatic ring. The condensation of an α -bromo ketone with malonic ester affords another means of introducing alkyl groups, for example: XII \longrightarrow XIII. The start-



ing materials are obtained by brominating the ketones prepared by the Friedel and Crafts reaction.

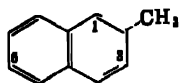
By suitable combination of these methods groups can be introduced at will at the positions C_1 , C_2 , C_3 , and C_4 , but substitution in the second terminal ring of phenanthrene is not subject to so much variation. Starting with α - or β -methylnaphthalene it is necessary to effect a substitution and cyclization in the unmethylated ring, as indicated by the dotted lines in formula XIV. In the case of α -methylnaphthalene, however, the reac-



tions with succinic anhydride and with acyl halides yield exclusively the 4-derivatives, the ortho-para directing methyl group promoting homo-nuclear substitution. The direct route being closed, Haworth employed as starting material the methylnaphthylamine sulfonic acid XV. After hydrolysis of the acid group, the group at C_6 was replaced by a nitrile group and a Grignard reaction then afforded 5-acetyl-1-methylnaphthalene (XVI). With this substance it was possible to build on a ring in the

desired 5,6-position and to introduce alkyl groups into the new ring by the above methods.

With β -methylnaphthalene the situation is more favorable than might have been anticipated from the fact that the hydrocarbon is nitrated and brominated almost exclusively in the 1-position. Sulfonation, however,



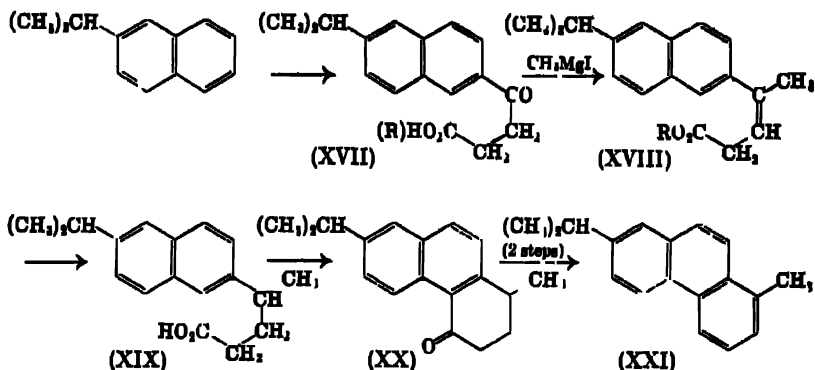
under conditions favoring β -substitution (high temperature), yields the 6-sulfonic acid as the chief product. The 1-acid would not be stable at the reaction temperature and the sulfonic acid group probably avoids the β -position C₂, partly because this is no true ortho position⁷² and partly because of the steric factor. The Friedel and Crafts reaction is also subject to steric influences, and the course of substitution is often dependent upon the temperature, the nature of the carbonyl reagent, and the character of the solvent. Although acyl halides condense with β -methylnaphthalene in carbon bisulfide solution almost exclusively in the 1-position, Haworth found that with succinic anhydride in nitrobenzene solution at a low temperature it was possible to obtain the 6-substituted derivative in yields as high as 79%. Although an evaluation of the different factors has not yet been made it appears probable that the solvent is of considerable importance and that nitrobenzene definitely favors β -substitution. Studying the action of acetyl chloride on naphthalene in nitrobenzene and in benzene, Rivkin⁷³ obtained chiefly the β -isomer in the former case and a mixture of equal parts of the α - and β -compounds in the latter instance. A possible explanation is that the bulky molecular compound from aluminum chloride, nitrobenzene, and the carbonyl component finds better spatial accommodation in the β -position than in the α -position. Possibly only a slight contributory influence of the solvent or of the carbonyl reagent is sufficient to alter greatly the course of substitution. Even with phthalic anhydride in tetrachloroethane solution, β -methylnaphthalene and 2,3-dimethylnaphthalene yield some products of heteronuclear substitution.⁷⁴

Making use of the above discovery, Haworth succeeded in synthesizing retene through the series of reactions: XVII \longrightarrow XXI. It is necessary to hydrogenate the unsaturated acid corresponding to XVIII prior to ring closure, as the unsaturated compound is not suitable for

⁷² Fieser and Lothrop, *J. Am. Chem. Soc.*, **57**, 1459 (1935).

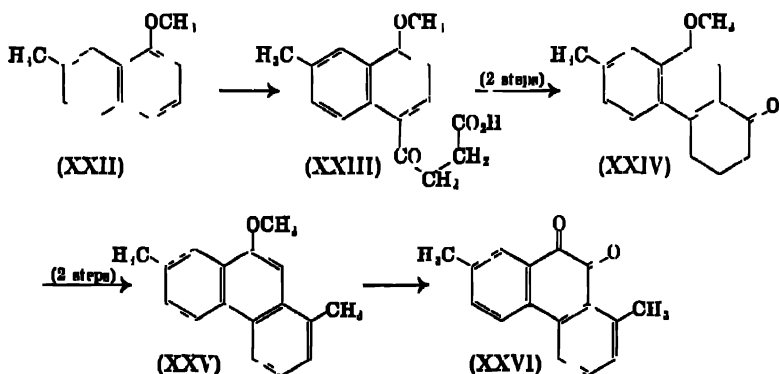
⁷³ Rivkin, *J. Gen. Chem.*, U.S.S.R., **5**, 277 (1935).

⁷⁴ Fieser and Peters, *J. Am. Chem. Soc.*, **55**, 3342 (1933).



such a reaction. The ketone is not converted directly into the aromatic hydrocarbon but is reduced by the Clemmensen method and the resulting hydrocarbon is dehydrogenated with selenium. In another series Haworth substituted the ethyl for the methyl Grignard reagent in the reaction with the ester of the keto acid XVII and obtained 1-ethyl-7-isopropylphenanthrene, identical with "methylretene." Pimanthrene and "methylpimanthrene" were synthesized similarly, starting with β -methylnaphthalene.

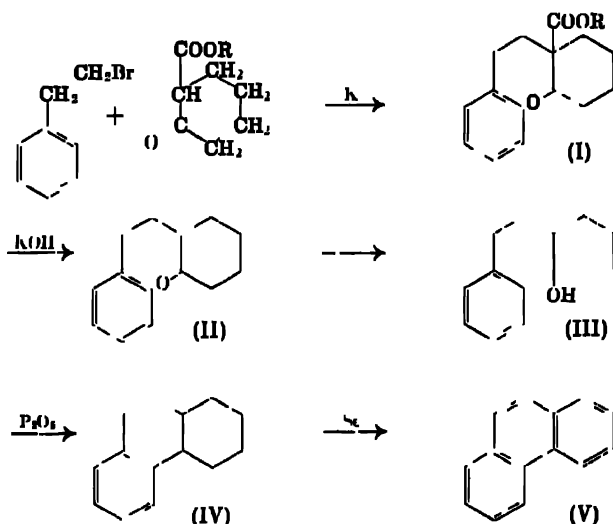
In independent work, Ruzicka and Waldmann⁷³ synthesized pimanthrenequinone by similar methods:



A methoxyl group was introduced in order to direct the succinic acid residue into the para position in the unmethylated ring of XXII. The keto acid XXIII was converted into the phenanthrene derivative XXV as above, and the methoxyl group was eliminated on oxidation to the quinone, XXVI.

⁷³ Ruzicka and Waldmann, *Helv. Chim. Acta*, 15, 907 (1932).

The Bardhan-Sengupta Synthesis. The synthetical work of Bardhan and Sengupta⁷⁶ in Calcutta, undertaken with the same objective as that of Haworth and at about the same time, was still incomplete when the dehydrogenation products from the resin acids had been fully identified by synthesis and by oxidation. The novel phenanthrene synthesis developed by the Indian chemists was to find its most fruitful applications in other fields. In the simplest example of the Bardhan-Sengupta synthesis β -phenylethyl bromide is first condensed with the potassium derivative of cyclohexanone-2-carboxylic acid ester. The sodium derivative of the β -keto ester is not suitable for the alkylation. The alkaline hydrolysis of the substituted β -keto ester (I) is accompanied by decarboxylation, giving the ketone II. This is reduced with sodium in moist

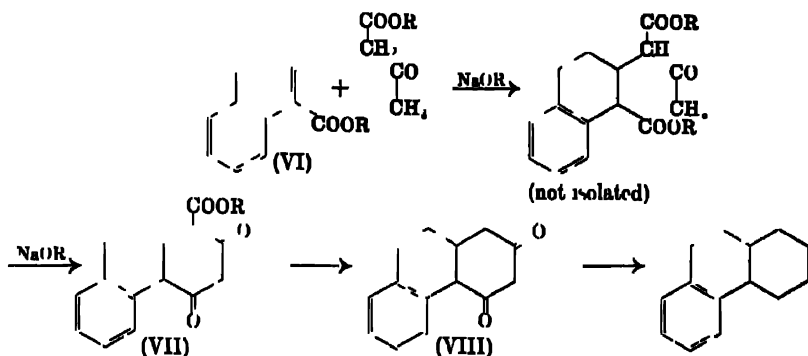


ether and the alcohol (III) is heated in vacuum with phosphorus pentoxide to effect dehydration and cyclization to octahydrophenanthrene (IV). Bardhan and Sengupta assumed that the ring is closed as the result of a direct elimination of water between the hydroxyl group and the benzene nucleus, but other observations to be presented below indicate that it is an unsaturated hydrocarbon formed by the simple dehydration of the alcohol which enters into the cyclization. The synthesis is completed by dehydrogenation with selenium. The structure of the octahydrophenanthrene (IV) was established by the following interesting synthesis, which constitutes an independent route to phenanthrene. Rabe⁷⁷

⁷⁶ Bardhan and Sengupta, *J Chem Soc*, 2520, 2798 (1932)

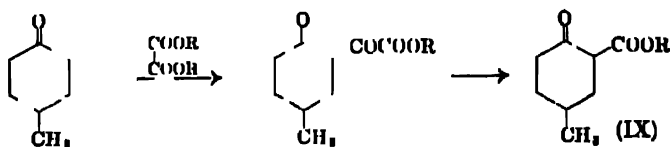
⁷⁷ Rabe, *Ber*, 31, 1896 (1898).

had submitted 3,4-dihydronaphthoic acid ester (VI) to the Michael reaction with acetoacetic ester and had found that the addition is followed by an ester condensation, with the closing of a new ring (VII). Hydrolysis of the ester gave a 1,3-diketone (VIII), and the Indian investigators found

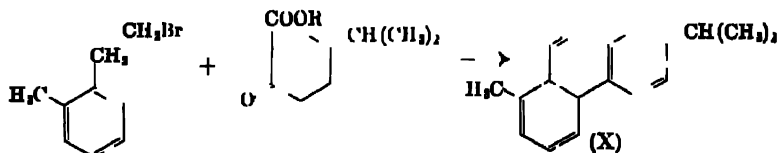


that on reduction by the Clemmensen method this yielded a hydrocarbon identical with their octahydride.

The beauty of the Bardhan-Sengupta synthesis is that it is capable of wide variation, as will be evident from the many applications to be described in later chapters. Substitution products of both of the reagents required for the initial condensation are readily available. Keto esters such as IX are conveniently prepared by the condensation of cyclohexan-

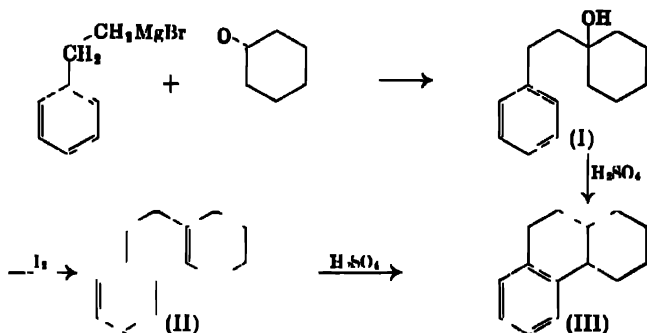


ones with oxalic ester. Both retene (X) and pmanthrene were synthesized by the new method, in the first case from the reagents indicated in the formulas.



The Perlman-Davidson-Bogert Synthesis. A still simpler route to phenanthrene, and one involving some of the same steps as in the method of Bardhan and Sengupta, was discovered independently by Perlman, David-

son and Bogert⁷⁸ at Columbia University. The first step in the synthesis consists in the Grignard condensation of β -phenylethyl magnesium bromide with cyclohexanone, giving the tertiary alcohol I. Under the



influence of concentrated sulfuric acid this undergoes "cyclodehydration" with the formation of octahydrophenanthrene (III), as in the Bardhan-Sengupta synthesis.⁷⁹ The cyclization in this case cannot result directly from the elimination of the elements of water, and the reaction was found to proceed in two steps. The unsaturated hydrocarbon II was prepared by brief treatment of the alcohol with 50% sulfuric acid or by catalytic dehydration with iodine, and it was found to undergo cyclization to III under the influence of concentrated sulfuric acid (also with aluminum chloride). The same unsaturated hydrocarbon undoubtedly is an intermediate in the Bardhan-Sengupta synthesis. The new synthesis is perhaps even more general in application and it provides a simpler means of obtaining the same type of intermediate for the cyclization. Bogert and his collaborators originally were interested in the synthesis of alkyl phenanthrenes for comparison with the hydrocarbons from abietic and *d*-pimaric acids,⁷⁹ but they have given attention chiefly to the use of the method for the synthesis of indanes⁸⁰ and ionenes,⁸¹ and to studies of the mechanism of the cyclization.⁸² Further evidence was obtained in the latter work that ring closure takes place by the isomerization of an unsaturated hydrocarbon, rather than by the direct dehydration of the alcohol. It was also found that hydrocarbons in which the double bond occupies different locations in the side chain can form the same cycle, if in varying yield.

Although the methods of Bardhan and Sengupta and of Perlman, Davidson and Bogert represent complete and original phenanthrene syn-

⁷⁸ Bogert, *Science*, **77**, 289 (1933). In a letter to the author Professor Bogert has requested that the work be cited under joint authorship, since that is the way in which the completed article will be submitted for publication.

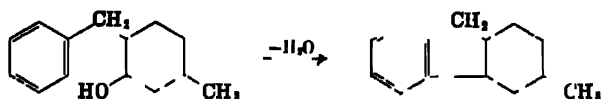
⁷⁹ Bogert and Stamatoff, *Rec. trav. chim.*, **52**, 594 (1933).

⁸⁰ Bogert and Davidson, *J. Am. Chem. Soc.*, **56**, 195 (1934).

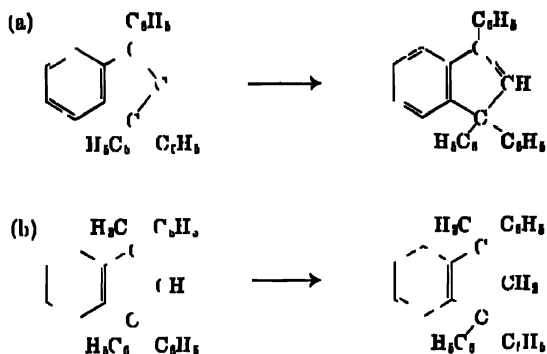
⁸¹ Bogert, Davidson and Apfelbaum, *ibid.*, **56**, 959 (1934).

⁸² Robin, Davidson and Bogert, *ibid.*, **57**, 151 (1935).

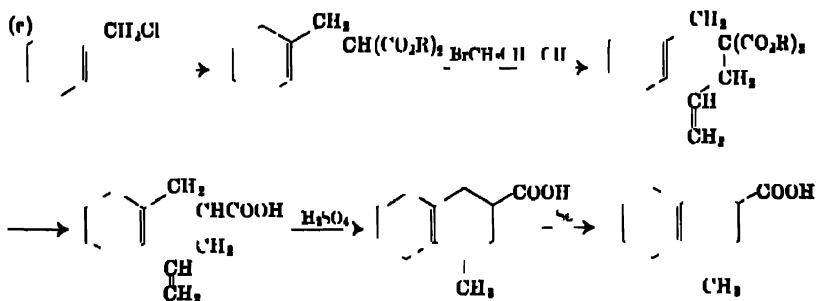
theses, the cyclization reaction upon which they are both based had been utilized previously by various investigators. As early as 1896 Wallach⁸³ obtained hydrofluorene derivatives by a process of cyclodehydration similar to that in the Bardhan-Sengupta synthesis, although he interpreted the reaction as a direct dehydration:



The conversion of certain aromatic glycols into phenyl indenenes was at first interpreted in the same way,⁸⁴ but it was later shown⁸⁵ that the cyclization is due to the isomerization of an unsaturated compound. Such an isomerization has been demonstrated in the cyclization of phenylated allenes into indenenes⁸⁶ (a) and of phenylated butenes into hydrindenenes⁸⁷



(b). The formation of a six-membered ring by a similar isomerization has been utilized by Darzens⁸⁸ in his general synthesis of naphthalene



⁸³ Wallach, *Ber.*, 29, 2982 (1896), *Ann.*, 305, 261 (1899)

⁸⁴ Orskhoff and Tiffeneau, *Bull. soc. chim.*, [4], 31, 253 (1922)

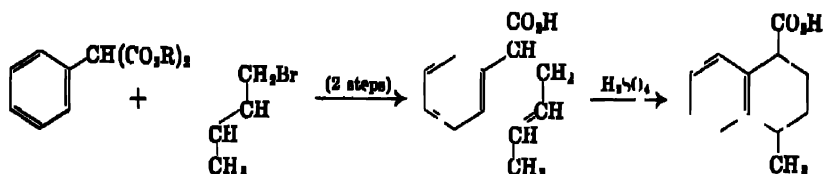
⁸⁵ Blum-Bergmann, *Ber.*, 65, 109 (1932), *J. Chem. Soc.*, 1020 (1935)

⁸⁶ Vorländer and Siebert, *Ber.*, 39, 1030 (1906), Kohler, *Am. Chem. J.*, 40, 217 (1908)

⁸⁷ E. Bergmann and Weiss, *Ann.*, 480, 40 (1930)

⁸⁸ Darzens and co-workers, *Compt. rend.*, 183, 748 (1926), 184, 33 (1927), 190, 1405, 1562 (1930), 194, 2056 (1932), 199, 1426 (1934), 200, 469 (1935)

derivatives (c). Extending the method to the phenanthrene series, Darzens prepared 1-methyl-3-phenanthroic acid from α -naphthylmethyl chloride.⁵⁹ The final product obtained⁶⁰ from β -naphthylmethyl chloride was 4-methylphenanthrene, the intermediate acid being decarboxylated in the course of the dehydrogenation with selenium. A cyclization of the same type is an essential feature of a further modification of the Darzens synthesis:⁶¹



⁵⁹ Darzens and Levy, *Compt rend*, **200**, 2147 (1935)

⁶⁰ *Idem*, *ibid*, **201**, 730 (1935)

⁶¹ *Idem*, *ibid*, **199**, 1131 (1934)

Chapter III

Cancer-Producing Hydrocarbons

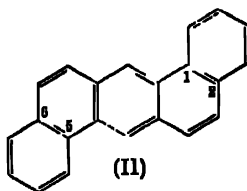
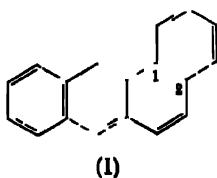
Of the diseases which have challenged the resources of the modern medical sciences one of the most baffling is cancer. As a cause of recorded deaths, cancer stands second only to heart disease, yet the origin of the disease is still a complete mystery and generally applicable methods either for the prevention of cancer or for its cure have been sought in vain. Treatment by surgery, X-ray, or radium is in a certain proportion of cases effective particularly in the early stages, but otherwise it offers only the possibility of alleviation. Generally cancer appears after the fourth decade, and the disease represents a condition of unchecked tumorous growth or cell proliferation. Normal cells multiply in an orderly manner and under a limiting control mechanism, but with malignant cells the proliferation is continuous and uncontrolled. The malignant cells continue to multiply and frequently migrate to other parts of the body where they set up secondary growths which often involve a vital organ, resulting in death. Cancer is not infectious, and it probably is not "hereditary" in the ordinary sense of the term, although with special, inbred strains of experimental animals susceptibility to cancer appears to be transmissible through inheritance. Some cancers, such as those produced by X-rays or by local chronic irritation, perhaps arise in the continued regenerative processes following injury to the epithelial cells, but the mechanism of the development of malignancy is still obscure. The many speculations on the way in which cells acquire the quality of malignancy remain without essential foundation.

Since any knowledge of the manner in which cancer originates may pave the way for the development of methods for combating the disease, it is a matter of great interest that certain forms of occupational cancer have been traced definitely to the source. It became apparent in the early part of the present century that workmen engaged in the distillation of coal tar are particularly prone to develop cancer of the skin. The obvious inference from the nature and the incidence of "tar cancer" that it arises from contact of the skin with chemical agents present in the tars or distillates was confirmed in 1915 when Yamagiwa and Ichikawa¹ succeeded in producing cancers by the long continued application of a coal

¹ Yamagiwa and Ichikawa, *Mittell med. Fakultät., Kaiser. Univ. Tokyo*, 15, 295 (1915).

tar distillate to the ear skin of rabbits. The further investigations of Bloch² indicated that the substances possessing carcinogenic activity are present in the high-boiling, neutral, nitrogen-free fractions. Kennaway³ found that cancer-producing tars can be obtained by the action of aluminum chloride on tetralin, by heating isoprene or acetylene in an atmosphere of hydrogen under pressure, and by other pyrolytic methods.

Following these observations, an intensive program of investigations was undertaken by Kennaway and his associates at the Research Institute of the Cancer Hospital, London. It seemed probable that the carcinogenic constituents of the tars are hydrocarbons, but all of the known constituents of the higher-boiling coal tar distillates were tested, using the mouse as the experimental animal, with completely negative results. The tests suggested that some unknown and probably rare constituent of the tar is responsible for the occupational cancer, but a search for such a substance was rendered difficult by the lack of a rapid method of biological assay. Tumors, whether spontaneous or induced, appear more rapidly in mice than in larger available animals possibly because the normal life span is shorter, but even with mice nearly a year may be required to obtain positive results. Progress was at first slow, but a means of guiding the search more rapidly was discovered at the Cancer Hospital by Mayneord and Hieger (1927-1930) in the use of fluorescence spectroscopy. Hieger⁴ observed that tars and oils known to produce cancer in test animals give characteristic fluorescence spectra with bands at 4000, 4180, and 4400Å. A number of hydrocarbons of known structure were submitted to optical examination in the hope of identifying the characteristic spectrum, and special attention was paid to the derivatives of anthracene, since fluorescence is one of the particularly striking properties of this group of substances. Anthracene itself has neither the band spectrum nor the activity of the substance of unknown structure, but 1,2-benzan-



thracene (I) was found to give bands rather similar to those of the carcinogenic tars but displaced in the direction of shorter wave length.

² Bloch and Widmer, *Arch. f. Dermat.*, 152, 529 (1920); Bloch and Draufuss, *Schwunns med. Wochenschr.*, 2, 1038 (1921).

³ Kennaway, *J. Path. Bact.*, 27, 233 (1924); *Brit. Med. J.*, 2, 1 (1925).

⁴ Hieger, *Biochem. J.*, 24, 505 (1930).

This significant observation led to the examination of a large number of polynuclear hydrocarbons, particularly those derived from 1,2-benzanthracene, for increase in the mass of the molecule should cause a shift of the spectral bands in the desired direction and might also lead to the development of cancer-producing properties. The desired compounds for the most part were prepared synthetically by J. W. Cook in a brilliant series of investigations⁵ which will be described below. Biological and optical investigations of the compounds were undertaken by E. L. Kennaway, W. V. Mayneord and I. Hieger. Among the substances studied at an early period of the work was 1,2,5,6-dibenzanthracene (II), a hydrocarbon which recently had become readily available through the development of a new method of synthesis (Clar, 1929). Although the correspondence of the fluorescence spectrum with that of the cancer-producing tars is far from precise, 1,2,5,6-dibenzanthracene was found to be an actively carcinogenic substance.⁶ Applied to mice by painting a 0.3 per cent solution in benzene onto the skin twice weekly, the hydrocarbon produced tumors in a large number of cases. For the first time it was shown that a pure hydrocarbon of known structure is capable of initiating cancerous growth.

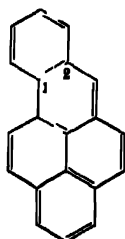
In subsequent studies the English investigators discovered carcinogenic activity in some of the simple alkyl derivatives of 1,2-benzanthracene, particularly those which resemble the active tetracyclic compound (II) in having substituents in the 5- and 6-positions. Only in very rare cases were hydrocarbons not related to 1,2-benzanthracene found to have carcinogenic properties, although a large number of such compounds were included in the study. Although the characteristic three-banded fluorescence spectrum did not prove to be an infallible criterion of carcinogenic activity, the general coincidence was sufficiently striking to warrant the hypothesis that the physiologically active constituents of the cancer-producing tars are identical with those responsible for the characteristic spectrum. The surmise proved to be correct, for Cook, Hewett and Hieger,⁷ making use of fluorescence spectroscopy, were able in 1933 to isolate a very actively carcinogenic constituent of coal tar. The quantity obtained from two tons of pitch by a lengthy process of distillation, solvent extraction, crystallization, and purification through the picrate was such as to indicate an original content of at least 0.003 per cent. The substance isolated was a previously unknown hydrocarbon for which the structure of 1,2-benzpyrene was established by synthesis. When the formula is

⁵ Cook, *J. Chem. Soc.*, 1057 (1930); 487, 499, 499, 3012, 2524, 2526, 3273 (1931); 456, 1473 (1932); Cook and Hewett, *ibid.*, 1408 (1933); Cook, *ibid.*, 1592 (1933), and later papers (see below).

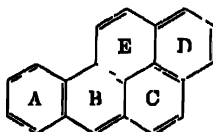
⁶ Kennaway and Hieger, *Brit. Med. J.*, 1, 1014 (1930).

⁷ Cook, Hewett and Hieger, *J. Chem. Soc.*, 395 (1933).

arranged in the manner shown on the right it is evident that the hydrocarbon contains the ring systems of 1,2-benzanthracene (ABCD), chry-



1,2-Benzpyrene



sene (ABED), and pyrene (BCDE). The absorption spectrum of 1,2-benzpyrene is definitely of the 1,2-benzanthracene type and appreciably different from those of chrysene or pyrene, although some features of the pyrene spectrum may be detected.⁸ The bond structure indicated above is in accordance with these observations.

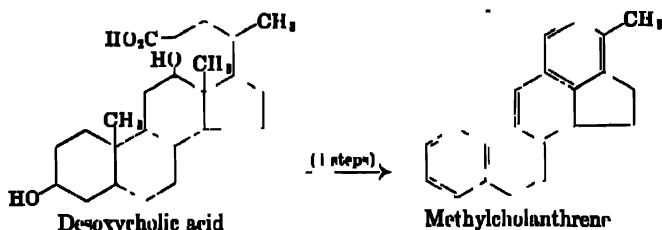
Pure 1,2-benzpyrene is considerably more potent than 1,2,5,6-dibenzanthracene in producing cancer in test animals, and the fluorescence spectrum is precisely that of the original tar except that the bands are more enhanced and better defined. Although the presence in the tar of other carcinogenic substances is not excluded, the evidence strongly indicates that the skin cancer prevalent among coal tar workers arises from prolonged contact with small amounts of this substance. Along with this hydrocarbon, the English investigators isolated 4,5-benzpyrene, perylene, and 1,2-benzanthracene, but none of these compounds is appreciably active in producing cancer in mice.

The discovery of 1,2-benzpyrene probably has a significance far deeper than that of disclosing the origin of tar cancer and pointing the way to proper protection in the industries in question. This substance and the synthetic hydrocarbons which resemble it in physiological action are not only capable of producing cancer of the skin, but they cause tumors to develop in any kind of tissue with which they come in contact. Epitheliomas result when application is made to the skin, but the subcutaneous injection of the hydrocarbons leads to the production of sarcomas (cancer of connective tissue). With the establishment of these facts it became possible for the first time to define positively conditions which can lead to the development of the disease without the participation of living agents: the acquisition by the organism of certain hydrocarbons related for the most part to 1,2-benzanthracene. The induced tumors strongly resemble those which arise spontaneously in the same animals

⁸ Mayneord and Roe, see *Proc. Roy. Soc., (London)*, B117, 336 (1935), A152, 209 (1935).

and it is quite possible that the malignancy is essentially the same. It is characteristic of many forms of ordinary cancer in humans that the disease can spread from one part of the body to another with the development of a similar lesion in the new location. This is known as metastasis. Secondary tumors produced by metastatic transfer of cells are composed of descendants of cells from the original tumor and they stand in marked contrast to the surrounding tissues. Although the sites usually chosen for the administration of the hydrocarbons are not particularly favorable for the development of secondary growths, definite metastases have been obtained in several instances. Clearly a knowledge of the mechanism whereby hydrocarbons of a particular molecular pattern are able to initiate malignant growth might be of value in devising methods for the control of the disease.

Cancer may originate in various different ways, and there was at first little reason to suppose that aromatic hydrocarbons play a part in the initiation of any but a particular form of occupational disease. The first evidence indicating that this view of the situation may require revision came as a result of the discovery of the highly potent carcinogenic hydrocarbon methylcholanthrene. This substance was obtained for the first time not by synthesis but as a degradation product of desoxycholic acid, one of the acids of the bile, by reactions which will be described in detail



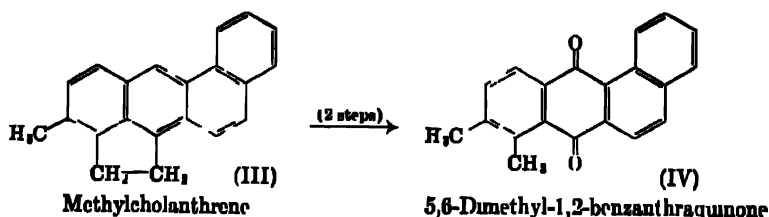
in Chapter IV. The degradation had been carried to within one step of completion by Wieland⁹ as early as 1925 and after the importance of the transformations to bile acid chemistry had become apparent (1932), Wieland and Dane¹⁰ in 1933 completed the series and isolated a yellow aromatic hydrocarbon of the anthracene group to which they assigned the name methylcholanthrene and the formula shown above.

When the formula is arranged as in III (page 86) it is evident that methylcholanthrene is a derivative of 1,2-benzanthracene having alkyl substituents at positions 5 and 6 (and 10), and consequently that it is closely related to the synthetic substances of carcinogenic activity which had been

⁹ Wieland and Schlichting, *Z. physiol. Chem.*, **150**, 267 (1925); Wieland and Wiedersheim, *ibid.*, **186** 339 (1930).

¹⁰ Wieland and Dane, *ibid.*, **219**, 240 (1933).

investigated at the Cancer Hospital in London. The relationship was fully appreciated by Cook, who had undertaken work on the problem ¹¹ even



before the appearance of the paper by Wieland and Dane, and in 1934 Cook and Haslewood ¹² reported that methylcholanthrene is a powerful carcinogenic agent. These investigators established the structure of the hydrocarbon by converting it by oxidation and decarboxylation into an anthraquinone (IV) which was identified by synthesis. The structure was further confirmed in 1935 by the synthesis of methylcholanthrene (page 105). In later work ¹³ methylcholanthrene was obtained from cholic acid, a still more abundant acid constituent of the bile. The laboratory preparation of the carcinogenic hydrocarbon from either desoxycholic acid or cholic acid involves the following steps: oxidation, hydrogenation, cyclization, and dehydrogenation. The fact that such transformations of substances normally present in the body can be realized by the brutal methods of the laboratory in no way indicates that the bile acids are converted into methylcholanthrene in the organism, and yet the reactions are all of types known to occur normally in the animal body. While proof is entirely lacking, it appears possible that many forms of cancer may originate in the metabolic production of methylcholanthrene or related substances from the bile acids, or perhaps from the sterols or sex hormones, of the body.

Observations suggesting a relationship between the sex hormones and the incidence of cancer will be discussed in Chapter V. In the following pages an account will be given of the synthesis and assay of the carcinogenic hydrocarbons.

Methods of Testing. In the early experiments of the English investigators the hydrocarbons were applied to the skin of mice in 0.3 per cent benzene solution twice weekly, the tumors produced being papillomas (benign tumors) or epitheliomas (skin cancers). When 1,2,5,6-dibenzanthracene was administered in this way, definite malignancy appeared for

¹¹ See Cook and Haslewood, *J. Am. Chem. Soc.*, 57, 1380 (1935).

¹² Cook and Haslewood, *J. Chem. Soc.*, 428 (1934).

¹³ Fieser and Newman, *J. Am. Chem. Soc.*, 57, 961 (1935).

the most part after 8-9 months. In later work ¹⁴ a solution of the hydrocarbon in lard, sesame oil, or other fatty medium was injected subcutaneously. Only one or two injections is required, and the tumors, which in this case are sarcomas, appear much more rapidly (after 4-5 months, in the case of 1,2,5,6-dibenzanthracene).

Ordinary ("stock") mice differ considerably in genetic constitution and in their susceptibility to induced or spontaneous cancer. With such animals individual results may not be closely reproducible and in order to gain an idea of the relative potency of different carcinogenic agents it is necessary to use large numbers of mice in making the tests. A valuable method of testing for malignancy in a tumor consists in transplanting a portion of the proliferated tissue into other mice (method of heterografts). If the tumor grows in the new host this affords convincing evidence of malignancy. The growth of the new tumor is considerably more rapid than that of the tumor originally induced by the hydrocarbon. With stock mice the results are irregular and only a positive result is conclusive. Andervont ¹⁵ found that tumors induced by hydrocarbons in pure-strain mice follow the genetic law of transplantation, that is, they will grow only in other mice of the same strain. The pure strains of mice are developed by brother-sister matings for at least twenty generations. With pure, inbred strains transplanted tissue almost invariably "takes" in case it is malignant and the results in general are far more reproducible. An active hydrocarbon will produce tumors in practically all of a group of mice if the animals live and if the material remains at the site of injection.

Pure-strain mice were employed in the recent experiments of Shear, ¹⁶ of the U. S. Public Health Service, who found that tumors can be induced somewhat more rapidly by the subcutaneous injection of crystals of the active hydrocarbons moistened with glycerol. Methylcholanthrene tumors were obtained by this method in 58 days. Shear also used pellets cast from a molten solution of the hydrocarbon in cholesterol, and he has suggested the use of pellets of graduated concentration as a means of determining the minimum dose required to produce a tumor. Preliminary results with 1,2,5,6-dibenzanthracene indicate that the amount required is considerably less than 1 mg. per mouse. ¹⁷

It would be of interest to have available for biological experimentation water-soluble carcinogenic agents which could be administered intravenously, and some advances in this direction have been reported. The

¹⁴ Burrows, Hieger and Kennaway, *Am. J. Cancer*, 16, 57 (1932).

¹⁵ Andervont, *U. S. Public Health Service Reports*, 49, 620 (1934); 50, 1211 (1935).

¹⁶ Shear, publication in press.

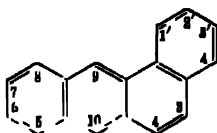
¹⁷ Private communication

sodium salt of 1,2,5,6-dibenzanthracene-9,10-endo- α,β -succinic acid¹⁸ and the salts of the 1,2,5,6-dibenzanthracene-choleic acid and methylcholanthrene-choleic acid¹⁹ give tumors when injected subcutaneously in mice, but further experiments have not been described.

Little work has been done with test animals other than mice. By applying a 0.3% solution of 1,2-benzpyrene to the skin of rabbits, Schürch and Winterstein²⁰ obtained a carcinoma in one of twelve rabbits after 400 days.

Comparison of the Carcinogenic Activity of Various Hydrocarbons. In the extensive tests conducted at the Cancer Hospital, Cook, Kennaway, and their associates²¹ found that methylcholanthrene and 1,2-benzpyrene are considerably more active than any of the other substances examined in producing cancer in stock mice. The high activity is shown both in the early appearance of tumors and in the high proportion of animals which develop tumors. 1,2-Benzpyrene is slightly less active than methylcholanthrene, and the latter substance is the most potent carcinogenic agent known. When applied to the skin of mice in dilute benzene solution, methylcholanthrene produces epitheliomas in about 5 months, 1,2-benzpyrene in about 5.7 months, and 1,2,5,6-dibenzanthracene in about 8 months. The same order of activity was found by Shear,¹⁸ using pure-strain mice and injecting the pure crystals.²²

The English investigators found that although 1,2-benzanthracene itself is practically inactive (1 tumor in 80 mice), it may be regarded as a potentially carcinogenic molecule for it acquires cancer-producing properties when alkyl groups are introduced at positions 5 or 6. 6-Methyl-1,2-benzanthracene is only feebly carcinogenic, the 5-methyl compound



is more potent, and there is a further increase on passing to the 5,6-dimethyl derivative (I), which is very nearly as effective in producing tumors as 1,2,5,6-dibenzanthracene. 5,6-Cyclopenteno-1,2-benzanthracene (II) closely resembles the 5,6-dimethyl derivative (I), and it is interest-

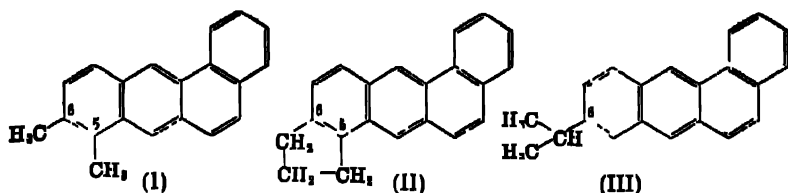
¹⁸ Cook, *J. Chem. Soc.*, 3273 (1931).

¹⁹ Fieser and Newman, *J. Am. Chem. Soc.*, 57, 861 (1935). See also Winterstein and Vetter, *Z. physiol. Chem.*, 230, 169 (1934).

²⁰ Schürch and Winterstein, *Z. physiol. Chem.*, 236, 79 (1935).

²¹ Cook, Hieger, Kennaway and Maynard, *Proc. Roy. Soc. (London)*, B111, 455 (1932); Cook, *ibid.* B111, 486 (1932); Barry, Cook, Haslewood, Hewett, Hieger and Kennaway, *ibid.*, B117, 318 (1933).

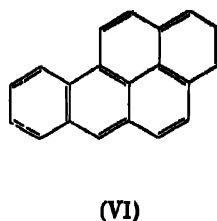
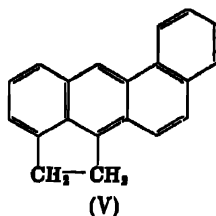
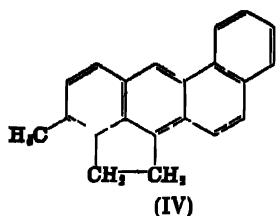
²² Confirmatory evidence of the high degree of potency of 1,2-benzpyrene has been presented by Maun and Laégeois, *Compt. rend. soc. biol.*, 115, 783 (1934); Roudot and Corbellini, *Atti accad. Lincei*, 21, 126 (1935); Schürch and Winterstein, *Z. physiol. Chem.*, 236, 79 (1935).



ing that approximately the same degree of activity is attained by the substitution at positions 5 and 6 of a benzene ring, a reduced five-membered ring, or two alkyl groups. The character of the alkyl group appears to be of importance for 6-isopropyl-1,2-benzanthracene (III) is definitely more active than the 6-methyl compound. A number of comparisons indicate clearly that a substituent at position 5 contributes more prominently to the development of carcinogenic properties than the same substituent at position 6. The high degree of specificity in the molecular structures associated with cancer-producing properties becomes apparent on considering the derivatives of 1,2-benzanthracene which have given negative, or practically negative, results. The list includes 3-, 4-, 7-, 2', and 3'-methyl-1,2-benzanthracene, the 2'6-, 2'7-, 3'6-, and 3'7-dimethyl derivatives, and the 3-, 7-, and 10-isopropyl derivatives. As far as the comparisons have been pursued, there is a remarkable regularity in appearance of carcinogenic activity among the 5,6-substituted compounds and in the absence of activity when the substituents occupy almost any other positions.

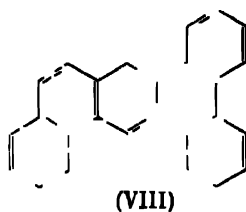
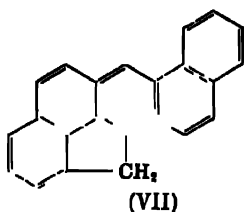
That the 5- and 6-positions are particularly favorable for the development of cancer-producing properties is further indicated in tests with a series of pentacyclic hydrocarbons derived from 1,2-benzanthracene by the attachment of an additional aromatic ring in the 2',3', 3',4', 3,4-, 5,6-, 6,7-, and 7,8-positions. Of these six isomers only 1,2,5,6-dibenzanthracene has shown pronounced carcinogenic activity. Of other hydrocarbons containing five condensed aromatic rings in the molecule only 1,2-benzpyrene has given rise to tumors. 4,5-Benzpyrene, perylene, picene, 2,3,6,7-dibenzanthracene, and 3,4,5,6-dibenzphenanthrene have given negative results.

As pointed out above, methylcholanthrene (IV) can be regarded as a 5,6-dialkyl-1,2-benzanthracene with an additional alkyl substituent at C_{10} . From the high potency of the hydrocarbon it would appear that the substitution in the meso position C_{10} is an important feature in the structure. Whether or not the five-membered ring is equivalent to two alkyl groups remains to be determined. The methyl group at C_6 in the 1,2-benzanthracene system does not appear to be important, for the



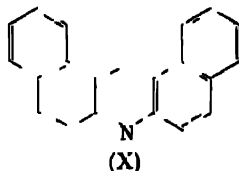
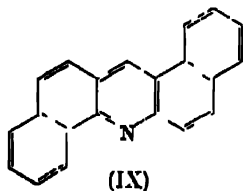
parent hydrocarbon cholanthrene (V) has about the same degree of activity as methylcholanthrene (Shear ¹⁸). It is interesting that cholanthrene and 1,2-benzpyrene (VI) are both 1,2-benzanthracene derivatives with an additional ring connected to a meso carbon atom of the parent hydrocarbon.

1',9-Methylene-1,2,5,6-dibenzanthracene (VII) has the cholanthrene ring system, but the added aromatic ring produces a great diminution in



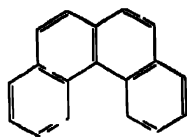
the carcinogenic activity for the hydrocarbon resembles 1,2,5,6-dibenzanthracene more closely than it does cholanthrene. Similarly, the addition of one or more aromatic rings to the 1,2,5,6-dibenzanthracene system, for example as in VIII, results in a loss of cancer-producing properties. Even simple substitution products of 1,2,5,6-dibenzanthracene show a reduced activity as compared with the parent hydrocarbon. The list of compounds examined includes the 2'- and 3'-methyl derivatives, the 9-amino, 9-methoxy, 9,10-dibenzyl, and the 4',3'-dimethylene compounds, as well as the octahydride, the 9,10-dihydride, and the 9,10-quinone. The last two cases are particularly interesting in connection with the perplexing question of the manner in which the aromatic hydrocarbon is able to initiate the condition of malignancy. If 1,2,5,6-dibenzanthracene acts by virtue of some chemical transformation in the body this would be expected to involve an oxidation or a reduction of the substance, and yet the first products of the chemical oxidation and reduction of the hydrocarbon both show diminished, rather than enhanced, activity. In the 1,2-benzpyrene series, Schürch and Winterstein ²⁰ found that the 3' (or 2'?) -methyl derivative and the 1',2',3',4'-tetrahydride are less active than the parent hydrocarbon.

Various heterocyclic isologues of the higher aromatic hydrocarbons have been examined by the English workers and definite if feeble carcinogenic activity was discovered in 1,2,5,6-dibenzacridine (IX) and 3,4,5,6-



dibenzacridine (X). Applied to mice in 0.3 per cent benzene solution, these substances produced tumors after about 13 months. That compound IX is carcinogenic is in keeping with the close structural relationship to the corresponding tetracyclic hydrocarbon, but it is rather surprising that the isomer should display similar properties.

All of the carcinogenic agents thus far discussed contain the 1,2-benzanthracene ring system (or its heterocyclic equivalent), but it appears that this is not an entirely essential feature of structure. It is true that very few cancer-producing substances have been discovered which are not related to 1,2-benzanthracene, although a great many such compounds have been submitted to tests. The following compounds, for example, have given entirely negative results: naphthalene, triphenylene, fluoranthene, chrysfluorene, and benzanthrene. Evidence of very slight carcinogenic power has been obtained with chrysene²¹ and pyrene, but it is not yet certain that the effects are not due to the presence of impurities.²⁴ On the other hand 3,4-benzphenanthrene, a hydrocarbon not related to the anthracene derivatives, possesses considerable carcinogenic activity. The substance acts slowly (benzene solution), but after about 12 months it produces tumors in a large proportion of the animals. It is the simplest carcinogenic substance yet encountered. Since the phenanthrene ring



3,4-Benzphenanthrene

system is contained both in this compound and in the 1,2-benzanthracene derivatives, it might be supposed that this is an essential structural feature, but the evidence available does not substantiate this view. Morton,

²¹ Bottomley and Twort, *Am J Cancer* 21, 791 (1934), Barry, Cook and others, *Ref* 21

²⁴ Barry, Cook and others, *Ref* 21, Schüch and Winterstein, *Ref* 20

Clapp and Branch²⁵ have reported the production of tumors in mice by the application of solutions of triphenylbenzene and tetraphenylmethane. Rather large doses were required and the tumors appeared only in a year's time, but they were of the characteristically malignant type. Clearly these substances are wholly unrelated to the condensed-ring hydrocarbons characterized by the English investigators. There may well be still other groups of compounds capable of initiating malignant growth.²⁶ In this connection reference may be made to another form of occupational disease, namely cancer of the urinary bladder (the so-called "aniline cancer").²⁷ Some agent or agents of nature still entirely unknown, but which appear to be associated with the manufacture of certain dyestuff intermediates, seem to be capable of inducing cancer of the bladder among workmen.

The observation that certain substances are capable of opposing the action of carcinogenic agents has been reported by Berenblum.²⁸ The production of tumors by potent tar fractions or by 1,2,5,6-dibenzanthracene was almost completely inhibited by the application of small quantities of mustard gas. The inhibition appears to be due to some local action on the tissues, rendering them refractory to the carcinogenic agents, for the refractory state appears almost as soon as the application is made and subsides soon after the treatment is discontinued. Mustard gas clearly does not react in any way with the carcinogenic agent, but merely modifies for a brief period the susceptibility of the tissues.

Of the known carcinogenic hydrocarbons perhaps methylecholanthrene is the most interesting, both because it is the most powerful agent yet discovered and because of the possibility that it represents a type of compound produced in the body as a product of abnormal metabolism. It should be expressly noted that this idea has neither been proven nor disproven, and that although it appears worthy of intensive investigation, the hypothesis may actually be quite far afield. Also entirely unknown is the mechanism whereby certain hydrocarbons start normal cells on a career of malignancy. If a chemical reaction is involved, the nature of the change is entirely obscure. Most of the carcinogenic hydrocarbons are more susceptible to oxidation than to other reactions, but there is no evidence that an oxidation is involved. The high degree of specificity in structure among the derivatives of 1,2-benzanthracene is difficult to reconcile with the hypothesis that an agent of a certain reducing intensity is required to initiate malignant growth, and this same specificity probably

²⁵ A. A. Morton, Clapp and Branch, *Science*, **82**, 134 (1935).

²⁶ Browning, J. B. Cohen, Cooper, Ellingworth and Guilbranson [*Proc. Roy. Soc. (London)*, **B11**: 300 (1933)] have reported the development of sarcomatous growth following the injection into mice of 2-(*p*-aminotyryl)-6-(*p*-acetylamino)benzoylamino-quinoline methoacetate.

²⁷ For a review, see W. C. Hueper, *J. Ind. Hyg.*, **16**, 255 (1934).

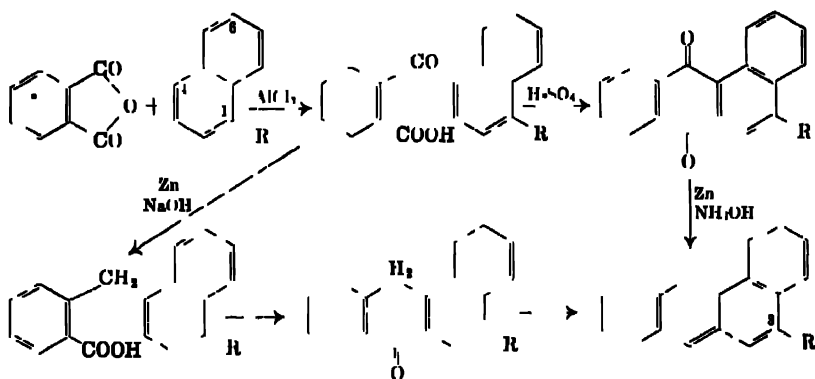
²⁸ Berenblum, *J. Path. Bact.*, **32**, 425 (1929); **34**, 731 (1931); **40**, 549 (1935).

rules out an explanation based upon the conception of a chronic irritation. Possibly the molecular dimensions and the surface activity of the substances are as important as their chemical characteristics.

METHODS OF SYNTHESIS

Although few distinctly new methods of synthesis have been developed in the search for cancer-producing hydrocarbons and in the attempt to define the limits of carcinogenic activity, several interesting applications and modifications have been made of the general methods already available. A discussion of these methods will illustrate the preparation of some of the compounds which have been of interest in the study.

The Phthalic Anhydride Synthesis. Although the phthalic anhydride synthesis offers a very convenient route to many of the simple substitution products of anthracene and anthraquinone, it is of only limited value in the preparation of *ang*-benzanthracenes and bis-*ang*-dibenzanthracenes. In one application of the synthesis phthalic anhydride is first condensed with a naphthalene derivative, and in the case of naphthalene itself ($R = H$) the keto acid resulting from the Friedel and Crafts reaction can be cyclized without difficulty and the hydrocarbon obtained by the reduc-



tion of the quinone. With some derivatives, acenaphthene for example,²⁹ ring closure proceeds with such difficulty on account of the presence of the unsaturated carbonyl group ortho to the position of substitution that it is necessary to reduce the keto acid prior to cyclization in order to obtain a satisfactory yield. 1-Methylnaphthalene ($R = CH_3$),³⁰ 1-isopropylnaphthalene,³¹ 1,6-dimethylnaphthalene,³² and acenaphthene³³

²⁹ Cook, *J. Chem. Soc.*, 1087 (1930)

³⁰ Scholl and Tritsch, *Momath.*, 32, 997 (1911), Cook, *Ref.* 29

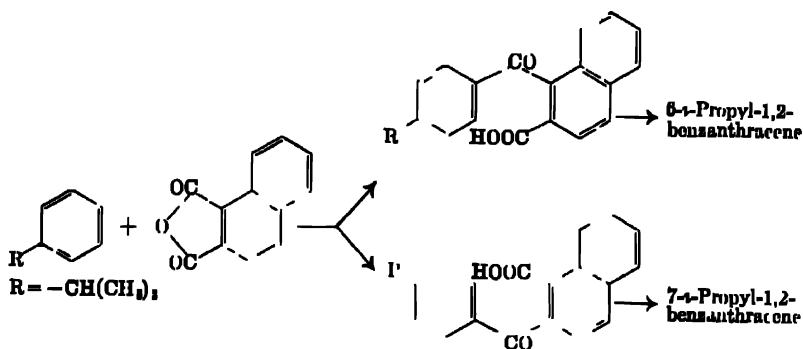
³¹ Cook, *J. Chem. Soc.*, 456 (1932)

³² L. F. Fieser and M. Fieser, *J. Am. Chem. Soc.*, 55, 3342 (1933)

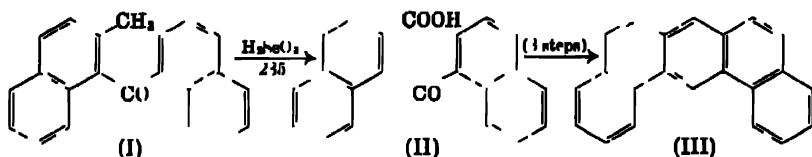
³³ Grube, *Ann.*, 327, 102 (1903)

react in the manner indicated for in each case an alkyl group at position 1 directs substitution at position 4. When the naphthalene nucleus contains only the more weakly orienting β -alkyl group the Friedel and Crafts reaction usually gives a mixture of isomers from which alkylated 1,2-benzanthraquinones can be prepared only with difficulty and in some cases by reactions involving rearrangements and the migration of alkyl groups.^{32,34} Where suitably substituted halogen compounds are available, the difficulties arising from the formation of isomers can be avoided by employing the Grignard synthesis, for substituted benzoyl- and naphthoyl-benzoic acids can be obtained in 70-80% yield by the action of aryl magnesium halides on phthalic anhydride.³⁵

An alternate procedure is to use 1,2-naphthalene dicarboxylic acid anhydride and a benzene derivative as the components in the synthesis, and this method has been used by Cook³¹ for the preparation of the carcinogenically active 6-isopropyl-1,2-benzanthracene. The unsymmetrical anhydride can react in two ways,³⁶ however, and in Cook's synthesis it was necessary to separate the isomeric keto acids and to establish their structures by oxidation



Closely related to the phthalic anhydride synthesis is the method used by Cook³⁷ for the preparation of 1,2,7,8-dibenzanthracene. The ketone (I) resulting from the condensation of α -naphthoyl chloride with



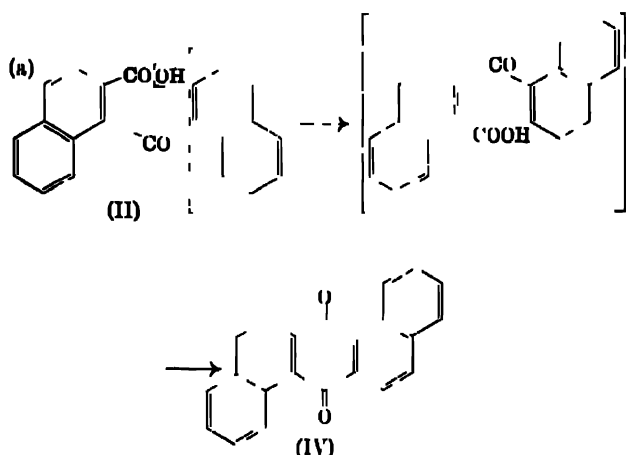
³¹ Ivers and Peters, *J. Am. Chem. Soc.*, **54**, 4712 (1932)

³² Weismann, E. Bergmann and F. Bergmann *J. Chem. Soc.*, 1967 (1915)

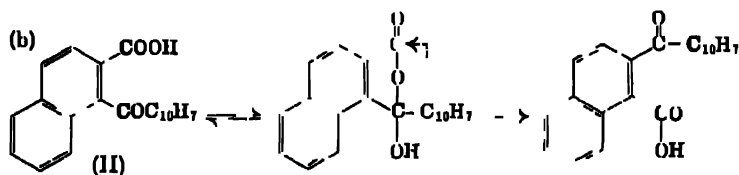
³³ Waldmann *J. prakt. Chem.*, **127**, 195 (1930) **131**, 71 (1931)

³⁴ Cook, *J. Chem. Soc.*, 1472 (1932)

β -methylnaphthalene was oxidized to the keto acid II by a method which unfortunately is not one of general application. The carbonyl group of II was reduced, the product was cyclized and the anthrone was reduced to the hydrocarbon III. The reduction of the keto acid prior to cyclization was in this case a matter of prime importance not because the cyclization of the acid II could not be accomplished but for the reason that a molecular rearrangement occurred in the course of the dehydration. Instead of the expected 1,2,7,8-dibenzanthraquinone, the keto acid on treatment with phosphorus pentoxide in nitrobenzene solution yielded 1,2,5,6-dibenzanthraquinone as the sole product. The rearrangement (a) must involve an exchange of position between the α -naphthyl radical and the hydroxyl of the carboxylic acid group. Cook suggested

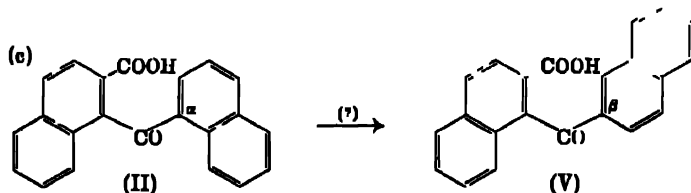


that the remarkable rearrangement proceeds through the lactol form of the keto acid, and the reaction becomes more understandable when formulated on this basis (b). It is worthy of note that the



α -naphthyl radical retains its structure during the migration and does not appear as a β -naphthyl group. In another case studied by Cook it is seen that a β -naphthyl radical can migrate without change in the position of the combining valence. It might appear that an equally reasonable

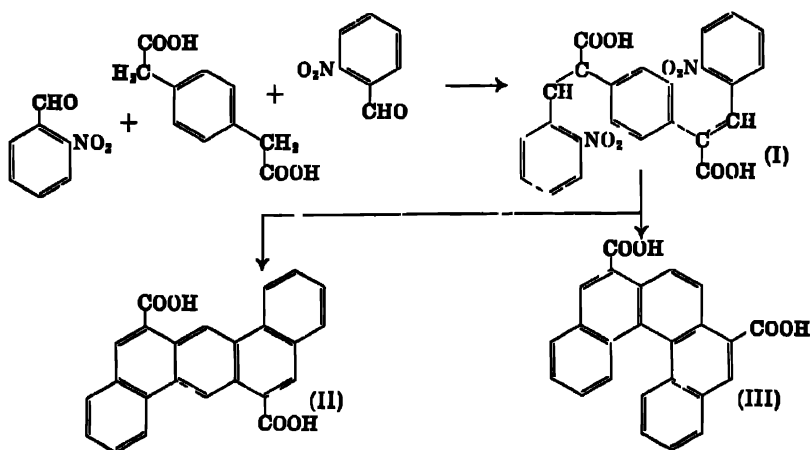
course for the rearrangement would consist in the migration of the substituted aroyl group from the α - to the β -position in the naphthalene nucleus (c). This view is untenable, however, because the keto acid V



was prepared synthetically and found to yield on dehydration not only the expected 1,2,5,6-dibenzanthraquinone but the isomeric 1,2,6,7-quinone and the 1,2,7,8-quinone. The first of these may arise from ring closure of the acid V at a β -position, but the second must be produced by way of a molecular rearrangement.

The occurrence of molecular rearrangements in the course of the cyclization of *o*-benzoylbenzoic acids was first observed by Hayashi³⁸ in 1927, but the true nature of the intramolecular change only became apparent as the result of Cook's investigations of 1932.

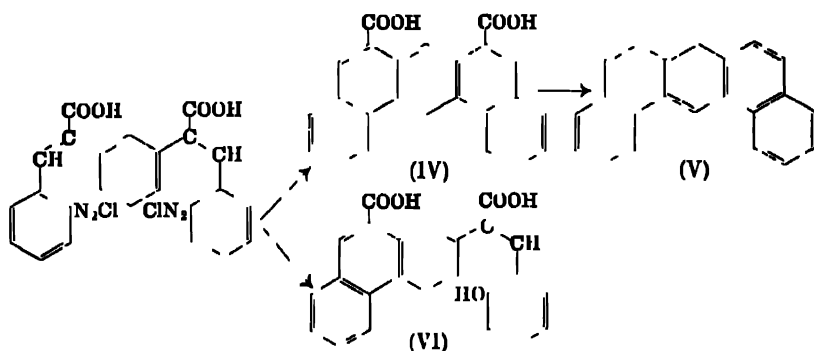
The Pschorr Synthesis. Since the phenanthrene nucleus is present in nearly all of the hydrocarbons known to possess carcinogenic activity, syntheses involving the formation of this three-ring system can be used for a few of the preparations. The Pschorr synthesis has been of service in obtaining some of the higher polynuclear types and indeed it was used in the first recorded synthesis of the important 1,2,5,6-dibenzanthracene.³⁹



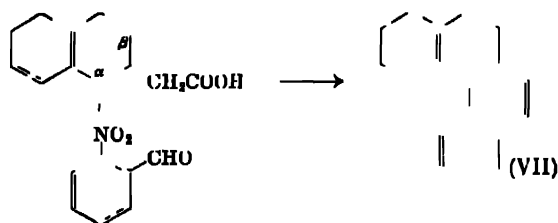
³⁸ Hayashi, *J. Chem. Soc.*, 2515 (1927), 1513, 1520, 1524 (1930).

³⁹ Wetsenböck and A. Klinger, *Monatsh.*, 39, 315 (1918).

p-Phenylenediacetic acid was used as the starting material and the synthesis involved a double ring closure of the initial condensation product. Cyclization of I took place in both possible directions, however, and there was obtained after decarboxylation of II and III a mixture of 1,2,5,6-dibenzanthracene and 3,4,5,6-dibenzphenanthrene. The formation of isomers coupled with rather poor yields and the fact that the starting materials are none too readily available greatly limits the usefulness of this type of Pschorr synthesis. Another difficulty was encountered by Cook³⁷ in the synthesis of 1,2,7,8-dibenzanthracene (V) from *m*-phenylenediacetic acid. Only a very small yield of the desired acid, IV, was obtained, for the chief reaction consisted in the hydrolysis of one of the diazonium salt groups, giving VI.



Although the Pschorr method has many disadvantages it has furnished a means of obtaining the cancer-producing 3,4-benzphenanthrene



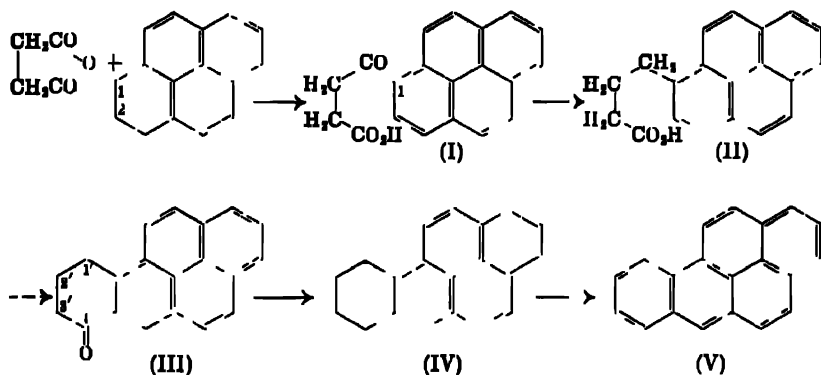
(VII). In correcting earlier work⁴⁰ on the subject, Cook⁴¹ showed that ring closure occurs at both the α - and β -positions of the naphthalene nucleus and that 1,2-benzanthroic acid is formed along with 3,4-benzphenanthroic acid. The earlier investigators had mistaken 1,2-benzanthracene for 3,4-benzphenanthrene. The β -naphthylacetic acid required

⁴⁰ Weitsenbock and Lieb, *Monatsh.*, 33, 504 (1912), F. Mayer and Oppenheimer, *Ber.*, 51, 510 (1918).

⁴¹ Cook, *J. Chem. Soc.*, 2524 (1931).

for the synthesis is obtained by means of a curious reaction discovered by Willgerodt in 1887: β -naphthylmethyl ketone is converted by yellow ammonium sulfide at 220° into the amide of the desired acid.

The Succinic Anhydride Synthesis. The general method of synthesis which Haworth found so useful for the preparation of alkylphenanthrenes (page 71) was found by Cook and Hewett⁴² to furnish a convenient route to the important 1,2-benzpyrene, a carcinogenic constituent of coal tar. The synthesis from pyrene is indicated in the formulas I-V. Because of the interest in obtaining supplies of the hydrocarbon for biological experimentation, the reactions have been studied in further detail by other investigators.⁴³ Cook and Hewett found that the Friedel and Crafts reac-

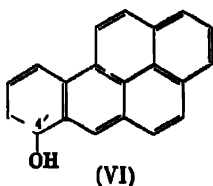


tion with pyrene proceeds smoothly in nitrobenzene solution giving a keto acid which probably is the 1-derivative (I), although this point has not been proved. The reduction of the keto acid by the Clemmensen method has not been accomplished, but the English workers found a satisfactory, if tedious, method in the use of zinc dust and ammonia. The cyclization to III was accomplished by heating II with anhydrous stannic chloride, but the procedure is much improved by condensing the acid chloride of II with stannic chloride (Fieser and Fieser). The cyclic ketone (III) can be converted directly in low yield into 1,2-benzpyrene (V) by treatment with selenium (C. and II) or by zinc dust distillation (F. and F.), but the best yield is obtained by preparing the tetrahydro compound IV by high-pressure hydrogenation and submitting the crude material to dehydrogenation (F. and F.). Using an equivalent amount of selenium, the ketotetrahydrobenzpyrene (III) can be converted in part into 4'-hydroxy-1,2-benzpyrene (VI), and with the use of sulfur the phenolic dehydrogenation product can be obtained in 19% yield.⁴⁴

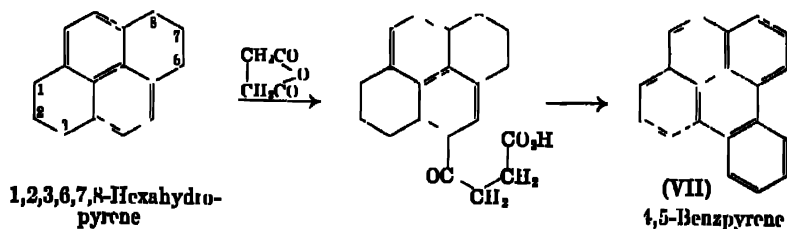
⁴² Cook and Hewett, *J. Chem. Soc.*, 398 (1933).

⁴³ L. F. Fieser and M. Fieser, *J. Am. Chem. Soc.*, 57, 782 (1935); Winterstein, Vetter and Schön, *Ber.*, 68, 1079 (1935).

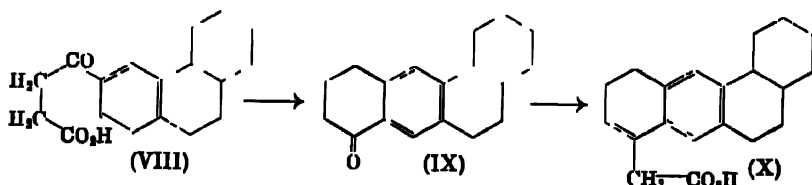
⁴⁴ Fieser, Hershberg and Newman, *J. Am. Chem. Soc.*, 57, 1509 (1935).



For the synthesis of 4,5-benzpyrene (VII), Cook and Hewett⁴³ employed a partially hydrogenated pyrene in order to change the orientation. Similar synthetic uses of *as*-octahydrophenanthrene have been inves-



tigated by Cook and Haslewood.⁴⁵ On condensation with succinic anhydride this yielded the keto acid VIII, which was converted by reduction



and cyclization into the ketone IX. A Reformatsky reaction of IX with ethyl bromoacetate afforded the acid X (with an isomer), and this was converted into cholanthrene, if in very poor overall yield, by saturation of the double bond, cyclization, reduction, and dehydrogenation.

The general method of Haworth has been used in two instances for the synthesis of alkylated 1,2-benzanthracenes.⁴⁶

The Elbs Synthesis. One of the most generally useful methods of obtaining polynuclear hydrocarbons is the Elbs anthracene synthesis, which consists in the pyrolysis of an ortho methyl diaryl ketone:

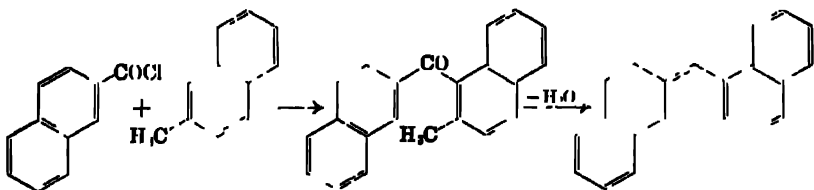


⁴³ Cook and Haslewood, *J. Chem. Soc.*, 767 (1935)

⁴⁶ Cook, *ibid.*, 1592 (1933); Cook and Haslewood, *ibid.*, 425 (1934).

The reaction is a curious one, involving in some manner not yet clear the elimination of the ketonic oxygen atom along with hydrogen atoms from the methyl group and the nucleus and the migration of hydrogen to the meso carbon atom. The first instance of this type of condensation was perhaps that reported in 1873 by Behr and van Dorp⁴⁷ who obtained anthracene on passing the vapor of *o*-tolylphenyl ketone over heated zinc dust. The anthracene, however, may have come from *o*-benzyltoluene formed on reduction of the ketone, for *p*-tolylphenyl ketone is reduced under similar conditions and the *o*-benzyl compound is known to yield anthracene on pyrolysis. Ador and Rilliet⁴⁸ observed the loss of water on the prolonged heating of certain *o*-methylbenzophenones but did not identify the products. Elbs⁴⁹ in a series of investigations carried out in the years 1884-1887, was the first to establish the course of the reaction and to study the application of the method to other compounds. A number of *o*-alkylated benzophenones were subjected to pyrolysis and it was found possible to obtain in this way several alkyl derivatives of anthracene. In general, however, the results were not very promising. The yields usually were in the order of 10 per cent and in some cases the reaction failed completely. The ketone was maintained at the boiling temperature until water was no longer given off or until the material was completely resinified, the period of heating varying from six hours to eight days. Condensing agents such as zinc chloride or phosphorus pentoxide either hindered the reaction or caused by-product formation. At a later date Seer⁵⁰ obtained yields of the order of 3 per cent from *m*-xylyltolyl ketone and *m*-xylylmesityl ketone after six days of heating.

This apparently unpromising reaction was not applied to any but phenyl ketones until 1929, when the pyrolysis of the dinaphthyl ketone obtained from β -naphthoyl chloride and β -methylnaphthalene was inves-



tigated by Clar⁵¹ and by Fieser and Dietz.⁵² It was found that the elimination of water can be completed in one-half hour and that 1,2,5,6-

⁴⁷ Behr and van Dorp, *Ber.*, **6**, 753 (1873); **7**, 10 (1874).

⁴⁸ Ador and Rilliet, *ibid.*, **11**, 390 (1878).

⁴⁹ Elbs and Larven, *ibid.*, **17**, 2817 (1884); Claus and Elbs, *ibid.*, **18**, 1797 (1885); Elbs and Olberg, *ibid.*, **19**, 409 (1886); Elbs, *J. prakt. Chem.*, **33**, 180 (1886); **35**, 465 (1887); **41**, 1, 121 (1887).

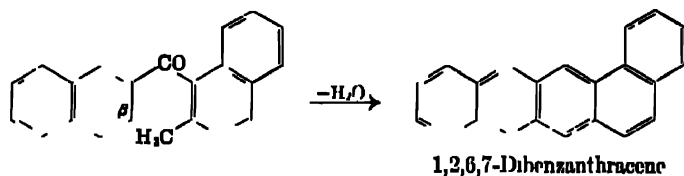
⁵⁰ Seer, *Monatsh.*, **32**, 143 (1911); **33**, 33 (1912).

⁵¹ Clar, *Ber.*, **62**, 350, 1878 (1929).

⁵² Fieser and Dietz, *ibid.*, **62**, 1827 (1929).

dibenzanthracene can be obtained easily in yields up to 32 per cent of the theoretical amount. The higher boiling point of the dinaphthyl, as compared with the diphenyl, ketones permits a higher temperature for the pyrolysis and this may contribute to the greater success of the reaction, but it is probable that a much more important factor is that in this case the methyl group condenses into a reactive α -position of the naphthalene nucleus. Because of this simple method of preparation 1,2,5,6-dibenzanthracene has been widely employed in studies of induced tumors.

In the above pyrolysis only about one-third of the ketone is converted into the hydrocarbon and it is not yet possible to account for all of the remaining material. One by-product⁵³ is β -methylnaphthalene, which probably arises from the hydrolytic action of water on the ketone at the high temperature of pyrolysis. Another is a chrysogen which gives to the crystals of 1,2,5,6-dibenzanthracene a beautiful yellow color which persists after innumerable crystallizations. It was not at first recognized that the color of the highly purified material is due to an impurity, but Cook⁵³ found that the hydrocarbon can be obtained in a colorless condition by shaking a solution of the yellow material in toluene with successive small portions of sulfuric acid, when the chrysogen is preferentially sulfonated. The process is effective, but wasteful of material. Cook⁵⁴ found that the colored impurity also can be removed by virtue of its more rapid reaction with maleic anhydride in boiling xylene solution, and suggested that the reactive chrysogen probably is 1,2,6,7-dibenzanthracene, formed by condensation at the β -position. That this infer-



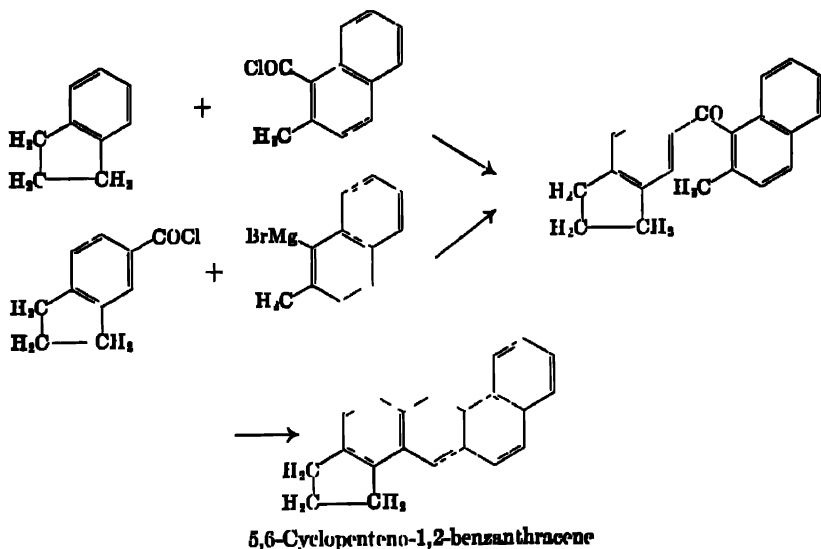
ence is correct was proved by Winterstein and Schön,⁵⁵ who isolated the chrysogen from the crude hydrocarbon by chromatographic adsorption analysis. Probably the most convenient method of preparing colorless 1,2,5,6-dibenzanthracene is to pass a solution of the yellow material in benzene through an adsorption tower of activated alumina, the material being recovered from the colorless filtrate. The yellow material forms a layer at the top of the column and the course of the colorless hydrocarbon can be followed by its fluorescence in ultraviolet light.

⁵³ Cook, *J. Chem. Soc.*, 497 (1931)

⁵⁴ Cook, *ibid.*, 3273 (1931); Cook, et al., *Proc. Roy. Soc. (London)*, B111, 469 (1932).

⁵⁵ Winterstein and Schön, *Z. physiol. Chem.*, 250, 146 (1931).

Although the yield in the Elbs synthesis leaves much to be desired even in the favorable case of the dinaphthyl ketones, the method is so simple and of such general application that it has found wide use in the study of carcinogenic activity. Of the various ways of preparing *o*-methyl ketones suitable for conversion into 1,2-benzanthracene derivatives, one consists in the Friedel and Crafts condensation of an aroyl chloride with β -methylnaphthalene, as above. Two other methods are illustrated by Cook's synthesis⁶⁶ of 5,6-cyclopenteno-1,2-benzanthracene.



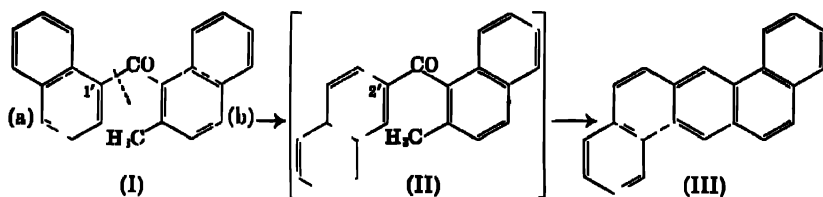
In the absence of strongly directive influences, the Friedel and Crafts reaction may lead to the formation of isomers and it is not as reliable as the other method. Another variation is to condense the Grignard compound with the nitrile, rather than with the acid chloride, and to obtain the ketone by the hydrolysis of the resulting ketimine.⁶⁷ Whether or not the ketone can be obtained in a condition free from isomers, the Elbs condensation usually can occur in two ways and a mixture of hydrocarbons often results on pyrolysis. This was true in the case cited, for 6,7-cyclopenteno-1,2-benzanthracene was isolated along with the main product. It is always necessary to purify carefully the product of an Elbs condensation and this is often effectively accomplished by the crystallization of the picrate, from which the hydrocarbon subsequently can be regenerated by distributing the molecular compound between benzene (or ether) and alkali. The addition compound with trinitrobenzene

⁶⁶ Cook, *J. Chem. Soc.*, 490, 2529 (1931).

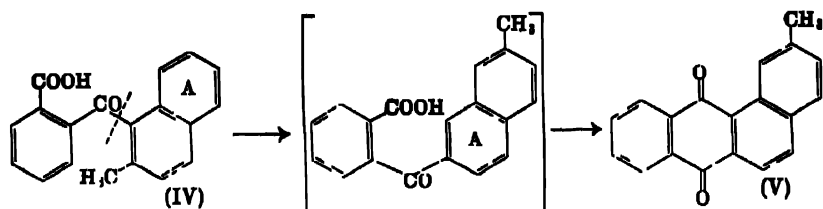
⁶⁷ Morgan and Coulson, *ibid.*, 2203, 2551 (1920).

sometimes has superior crystallizing properties and when this derivative is used for purification the hydrocarbon can be recovered by treatment with stannous chloride, removing the triaminobenzene as the water-soluble hydrochloride. It usually is necessary to furnish independent evidence of the structure of the reaction product. In the example above the hydrocarbon was oxidized with acid permanganate to an anthraquinone tetracarboxylic acid which proved to be identical with the acid obtained by a similar oxidation of 1,2,5,6-dibenzanthracene, the nicely crystalline and sharply melting tetramethyl ester being used for the comparison.

A further reason for exercising caution in assigning a structure to a condensation product is that molecular rearrangements often occur in the course of the Elbs reaction. The most notable case is that of the pyrolysis of the ketone from α -naphthoyl chloride and β -methylnaphthalene. When the reaction was first studied by Clar⁶¹ and by Fieser and Dietz⁶² it was

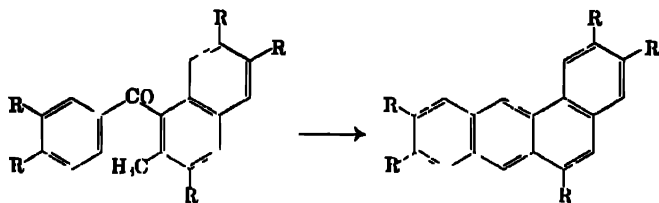


assumed that the hydrocarbon obtained was 1,2,7,8-dibenzanthracene, but Cook⁶³ proved that the substance actually is 1,2,5,6-dibenzanthracene. It appears that at the high temperature of the reaction (440°) the 2-methylnaphthoyl group (b) migrates from the 1'- to the 2'-position in the naphthalene nucleus (a), giving an isomeric ketone (II) which can condense in the manner indicated. The conversion of I into the hydrocarbon takes ten times as long as when II is used, presumably because time is required for the isomerization. The rearrangement of an α -substituted naphthalene to a β -derivative is not uncommon, and yet it is very odd that α, α' -dinaphthyl ketone is not isomerized at the temperature used for the above pyrolysis.⁶³ There is as yet no evidence that a rearrangement of the ketone I occurs *prior* to the condensation and the above formulation may be only a hypothesis of convenience. The same may be

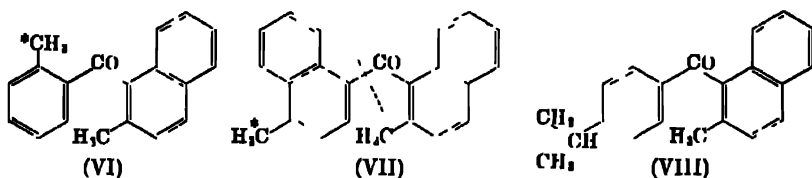


said of the somewhat similar rearrangements observed by Fieser and Fieser^{32,34} on heating alkylated naphthoylbenzoic acids such as IV (page 103) with sodium aluminum chloride. The rearrangement, whether prior to or during the ring closure, is from an α - to a β -position, but in this case the aryl group migrates into the unsubstituted ring (A).

Another source of uncertainty regarding the structures of the Elbs condensation products is that alkyl groups often do not withstand the high temperature of pyrolysis. In the reaction:



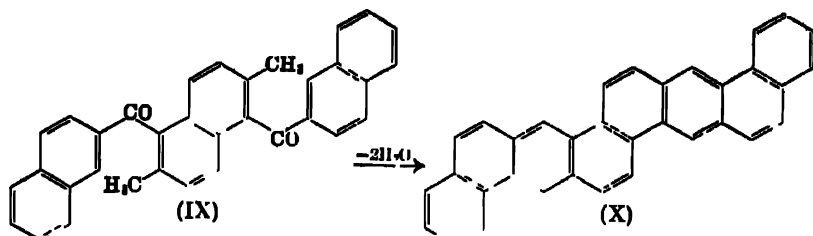
methyl groups situated in any of the five positions indicated by R appear at least in large part in the corresponding positions of the reaction product, but in several other cases on record a methyl group is eliminated in the course of the condensation. This is true of compounds VI³¹ and VII,³⁴



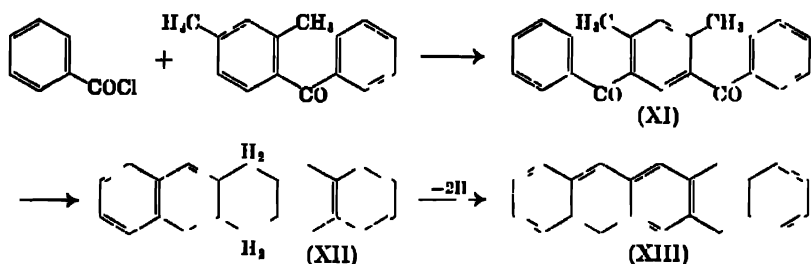
the starred groups being eliminated. In the second case there is also a rearrangement and the product is 1,2,5,6-dibenzanthracene. An alkyl group of more complex structure may suffer degradation, as has been shown in the case of the ketone VIII.³¹ The isopropyl group persisted to some extent, but it was partially converted in the course of the pyrolysis into a methyl group. Even when it is possible to isolate a hydrocarbon containing all of the original carbon atoms, it is almost invariably true that many crystallizations of the substance, preferably in the form of the picrate, are required to effect a complete purification. Homologues and isomers are probably present in the crude mixtures.

One extension of the synthetical method of Elbs is the application to diketones of suitable structure. Thus Fieser and Dietz³² converted the diketone IX, obtained from 2,6-dimethylnaphthalene and two moles of β -naphthoyl chloride, into 2,3,8,9-di-(1',2'-naphtho)-chrysene, X. Probably because both ring closures occur at reactive α -positions, the conden-

³¹ Cook, *J. Chem. Soc.*, 489 (1931).



sation proceeds very smoothly and the orange, very sparingly soluble octacyclic hydrocarbon melting at 500° is obtained in particularly good yield (52 per cent). Various dibenzoyldimethylnaphthalenes have been similarly condensed to hexacyclic hydrocarbons by Clar, Wallenstein and Avenarius,⁵⁹ and dimethyldiketones of the benzene series have been investigated also by Clar and his collaborators.⁶⁰ The most interesting outcome of this work was the synthesis of 2,3,6,7-dibenzanthracene,⁶¹ a deep blue hydrocarbon of such extraordinary reactivity that Clar suggested for it the structure of a diradical. The diketone (XI) required for the synthesis was isolated from a mixture of isomers resulting from the condensation of benzoyl chloride with *m*-xylylphenyl ketone. The product



of pyrolysis was a dihydride (XII) or a mixture of dihydrides, and the blue aromatic hydrocarbon (XIII) was obtained by boiling a solution of the material in nitrobenzene with phenanthrenequinone to effect the dehydrogenation. The formulation of the substance in terms of Kékulé rings provides an adequate interpretation of the properties, for this includes the unstable *o*-benzoquinonoid and 2,3-naphthoquinonoid groupings.

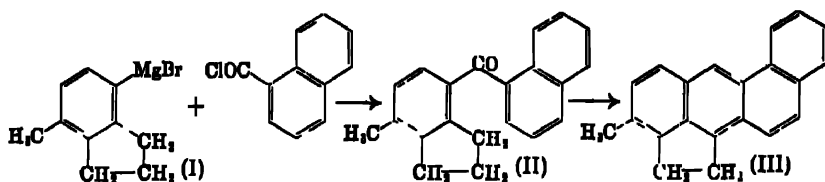
A modified Elbs condensation was employed by Fieser and Seligman⁶² in the synthesis of methylcholanthrene (III). The novel feature is that a methylene group of an alicyclic ring replaces the usual methyl group in

⁵⁹ Clar, Wallenstein and R. Avenarius, *Ber.*, **62**, 950 (1929).

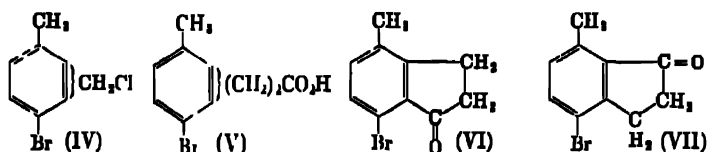
⁶⁰ Clar, John and Hawran, *ibid.*, **62**, 940 (1929); Clar and John, *ibid.*, **64**, 981 (1931).

⁶¹ Clar and John, *ibid.*, **62**, 3021 (1929), **63**, 2067 (1930).

⁶² Fieser and Seligman, *J. Am. Chem. Soc.*, **57**, 229, 942 (1935).

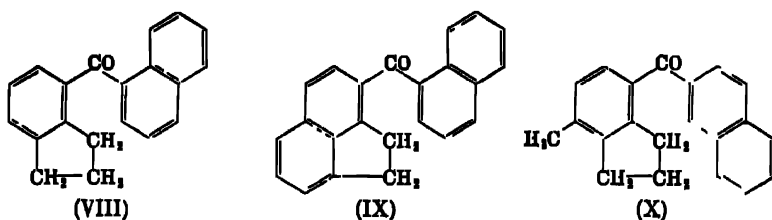


the condensation and consequently that a meso-substituted anthracene is obtained. In view of the low yields usually obtained in the ordinary Elbs reaction and the complications due to the elimination or degradation of alkyl substituents, it is rather surprising that the ketone II on pyrolysis gives very pure methylcholanthrene in about 45% yield. The 4-bromo-7-methylhydrindene required for the synthesis was obtained from the mixture of the two chloromethyl derivatives (IV) prepared from *p*-bromotoluene by the Blanc reaction (formaldehyde, hydrogen chloride,



zinc chloride). Following the condensation of the halide mixture with malonic ester and hydrolysis to V, cyclization gave an easily separated mixture of the hydrindones VI and VII. Both ketones yielded the desired hydrindene on reduction.

The modified Elbs reaction has given equally satisfactory results with other ketones having a five-membered alicyclic ring. Cholanthrene⁸³ and 1',9-methylene-1,2,5,6-dibenzanthracene⁸⁴ were readily obtained by the pyrolysis of the ketones VIII and IX, respectively. β -Naphthoyl-



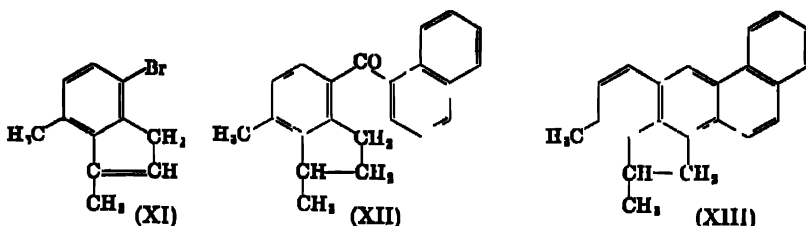
hydrindenes such as X also undergo ready intramolecular condensation and give pure hydrocarbons.

The method appears to be capable of considerable variation. For

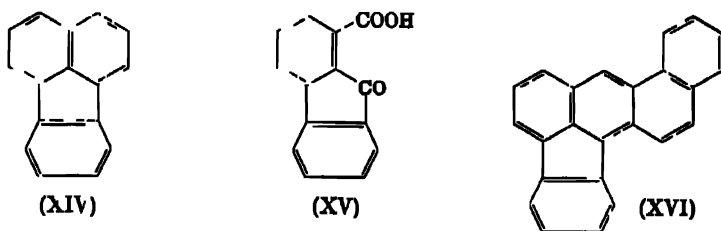
⁸³ Fieser and Seligman, *J. Am. Chem. Soc.*, **57**, 2174 (1935).

⁸⁴ Fieser and Hershberg, *ibid.*, **57**, 1681 (1935).

example the dimethylcholanthrene XIII was obtained⁶⁸ from 7-methyl-4-bromohydrindone-1 (VII, above), through the methyl Grignard reac-



tion product XI and the ketone XII. Fluoranthene (XIV) served as the starting material for the preparation⁶⁹ of another hydrocarbon (XVI)



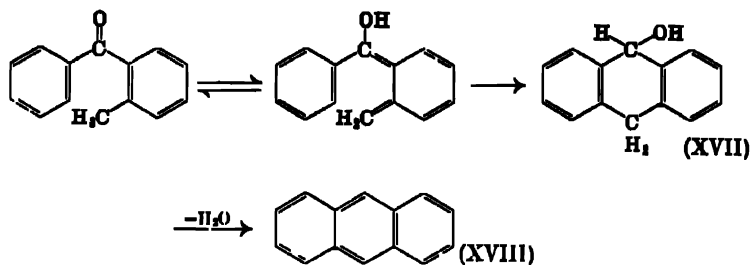
containing the cholanthrene ring system. Fluorenone-1-carboxylic acid (XV) is readily obtained by the oxidation of the coal tar hydrocarbon (XIV), and after reduction of the carbonyl group a condensation of the acid chloride with α -naphthyl magnesium bromide affords a ketone which yields XVI on pyrolysis.

Ketones having six-membered alicyclic rings in the ortho position to the carbonyl group undergo cyclodehydration on pyrolysis, but the reactions are accompanied by secondary changes⁶⁶. In particular, hydrogen atoms of the alicyclic ring in this case appear prone to migrate to other parts of the molecule.

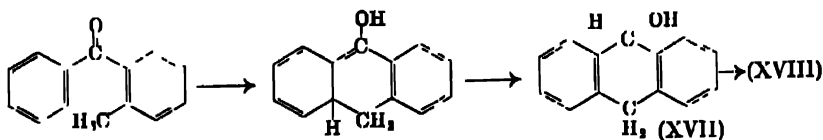
There is as yet no evidence available regarding the mechanism of the Elbs reaction, and the two alternate views which have been expressed are in the nature of speculations. Cook⁶³ has suggested that at the high temperature necessary for dehydration the ketone undergoes a tautomerization to a quinonoid structure. The ring closure then may consist in an intramolecular 1,4-addition to the unsaturated system of the enol. Dihydroanthranol (XVII) would be expected to lose the elements of water easily and yield anthracene. An alternate suggestion (Fieser and

⁶⁸ Fieser and Seligman, *J. Am. Chem. Soc.*, **57**, 1377 (1935).

⁶⁹ Fieser and Seligman, *ibid.*, **58**, 478 (1936).



Dietz⁵²) is that the reaction proceeds through an intramolecular 1,4-addition of the methyl group to the conjugated system of the ketone, after the manner of the forced Grignard additions to 1,4-systems of which the benzene nucleus forms a part:



Either mechanism accounts for the failure of chemical dehydrating agents to bring about the change and, since dihydronanthranol is assumed in each case to be an intermediate, both mechanisms account for the occasional isolation of anthrones from the reaction mixtures,^{34,57} for these may arise from the dehydrogenation of the intermediate in preference to its dehydration.

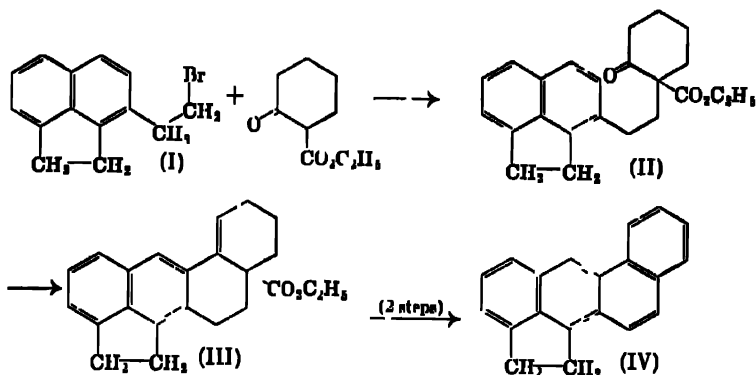
Other Methods. In investigating possible synthetic routes to hydrocarbons having the cholanthrene ring system, Cook, Haslewood and Robinson⁵⁸ studied the application to the problem of both the Bardhan-Sengupta and the Perlman-Davidson-Bogert methods, but met with success only in the first case. From the difficultly available 1-iodoacenaphthene,⁵⁹ β -1-acenaphthylethyl bromide (I) was prepared by the condensation of the Grignard reagent with ethylene oxide and conversion of the alcohol to the bromide. Condensation of I with the potassium derivative of ethyl cyclohexanone-2-carboxylate gave the β -keto ester II, which was cyclized to III by boiling 55% sulfuric acid. The ester III was very difficult to hydrolyze, but the corresponding acid was obtained by treatment with sodium ethylate at 180° and then dehydrogenated and decarboxylated over platinum black at 300°, giving cholanthrene, IV.

A synthesis of cholanthrene, more convenient than either this method or that employing *as*-octahydrophenanthrene as the starting material

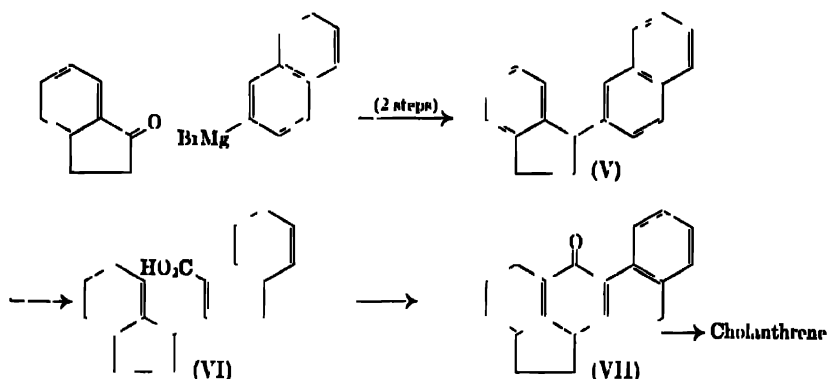
⁵² Morgan and Coulson, *J. Chem. Soc.*, 2551 (1929).

⁵⁸ Cook, Haslewood and (Mrs.) A. M. Robinson, *Ibid.*, 867 (1935).

⁵⁹ Morgan and Harrison, *J. Soc. Chem. Ind.*, 49, 413T (1930).



(page 99), was developed by Cook and Haslewood.⁷⁰ 1- β -Naphthylhydrindene (V) was obtained by the hydrogenation of the indene resulting from the condensation of hydrindone-1 with β -naphthyl magnesium



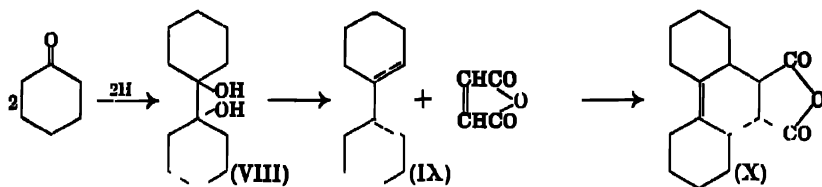
bromide. Bromination of the hydrocarbon V gave a resinous mixture, but, after removing compounds brominated in the five-membered ring with pyridine, carbonation of the Grignard reagent gave the acid VI. After cyclization with cold sulfuric acid, the anthrone VII was reduced to cholanthrene with zinc dust and alkali.

In concluding this survey of the methods of synthesis which have been found useful in the study of carcinogenic activity, mention may be made of two additional syntheses which have been introduced only recently but which bear some promise of future application. Both methods are specific for the synthesis of the phenanthrene ring system, and both are adaptations of the general reaction of Diels and Alder. Barnett,⁷¹ and

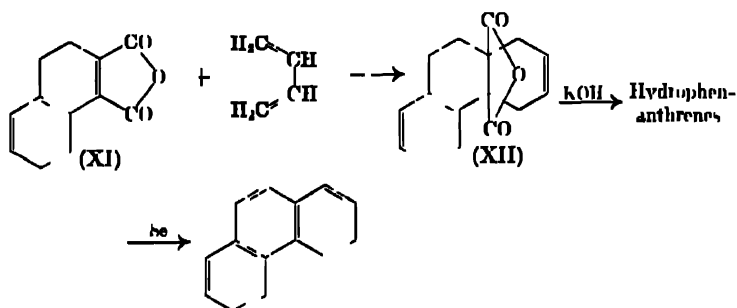
⁷⁰ Cook and Haslewood, *J. Chem. Soc.*, 770 (1935)

⁷¹ Barnett and Lawrence, *ibid.*, 1104 (1935)

later Adams,⁷² conceived the idea of employing as the diene a hydrocarbon (IX) readily obtained by the dehydration of the pinacol (VIII)



from cyclohexanone. The unsaturated hydrocarbon combines with maleic anhydride to give the hydrophenanthrene derivative X. The synthesis has been varied by the use of other cyclic ketones and by employing quinone, α -naphthoquinone, and acrolein as the second component, but the aromatization of the addition products has not yet been accomplished. In contrast to this method, Fieser and Hershberg⁷³ employed open-chain dienes and cyclic derivatives of maleic anhydride, such as 3,4-dihydronaphthalene-1,2-dicarboxylic acid anhydride (XI). The Diels-Alder reaction proceeds smoothly and the two tertiary bound carbonyl groups of the addition product XII can be eliminated by fusion with alkali. This gives a mixture consisting largely of tetra- and hexa-



hydrophenanthrenes from which pure phenanthrene can be obtained by dehydrogenation. Since substitution products and benzologues of the starting material (XI) are available by a convenient synthesis (page 221), the method may find general application; thus far it has been used only for the synthesis of simple derivatives of phenanthrene, 3,4-benzophenanthrene, and chrysene.⁷⁴

⁷² Gruber and R. Adams, *J. Am. Chem. Soc.*, **57**, 2553 (1935). See also Weidloch, *Z. anorg. Chem.*, **65**, 707 (1937).

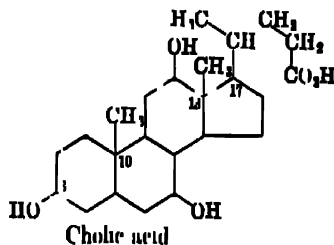
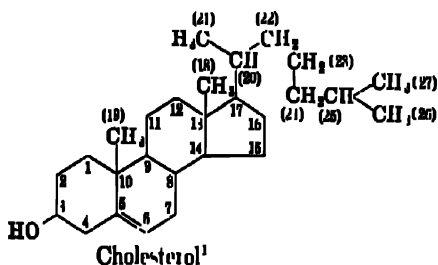
⁷³ Fieser and Hershberg, *J. Am. Chem. Soc.*, **57**, 1808, 2192 (1935).

⁷⁴ L. F. Fieser, M. Fieser and E. B. Hershberg, unpublished work.

Chapter IV

Sterols and Bile Acids

The unravelling of the complicated problem of the nature of the sterols and bile acids is one of the outstanding achievements of modern organic chemistry and one which has proved to be of the utmost importance to the progress of investigations in several related fields. The determination of the structures was an extremely difficult matter, but the acids of the bile and the sterols of plants and animals are substances of such importance in physiological processes that the investigations initiated over a century ago were pursued with fearless zeal and in the period 1932-1934 the major problems finally were settled in all the most essential details. From the evidence now available it is quite clear that cholesterol and cholic acid, the most important representatives of the two series of compounds, are correctly represented by the following formulas:



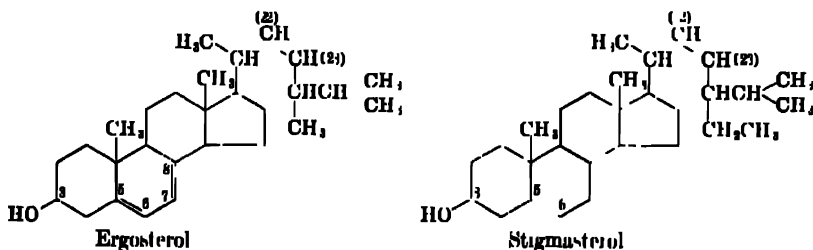
The two structures are remarkably similar. The ring system in each case is that of perhydro-1,2-cyclopentenophenanthrene, the secondary hydroxyl group of cholesterol occupies a position (C_3) corresponding to one of the three such groups of cholic acid, tertiary bound methyl groups are present in each case at C_{10} and C_{13} , and the side chains differ only in the length and in the nature of the terminal group. Before discussing the extensive series of investigations leading to the establishment of these formulas, it will be well to consider briefly the occurrence and properties of the more important members of the two groups of natural products.

DESCRIPTION

The Sterols. Along with the phosphatides, the sterols are regular constituents of animal and plant fats and oils. They are neutral and

¹ The rather irrational system of numbering originated as a compromise between the old and the new conceptions of structure.

comparatively stable substances which occur partly in the free condition and partly esterified with higher fatty acids. The usual method of isolation consists in subjecting the neutral, fatty fraction to hydrolysis with alcoholic alkali and extracting the unsaponifiable matter with ether or petroleum ether. Although the sterols are nicely crystalline compounds, the materials obtained from most sources are mixtures of closely related sterols of similar solubilities, and the separation often is a difficult matter. This is particularly true of the sterols of vegetable origin, the phytosterols, which occur in small amounts in all parts of plants and are found in relatively great abundance in seeds and pollen. Ordinary preparations of sitosterol (Gr. *sito*-, grain), the most widely distributed sterol of plants, have been found by R. J. Anderson and co-workers² to consist of mixtures of at least three optical isomers and to contain appreciable amounts of dihydrosito-sterols. The phytosterol mixture from Calabar bean contains stigmasterol and sitosterol, and that from yeast is composed of ergosterol and zymosterol. These substances are all of much the same composition as cholesterol (see table) and they are closely related to this substance in structure, as will be seen from the formulas



of the two most fully characterized members of the group. Ergosterol differs from cholesterol only in having an additional methyl group in the side chain (C_{24}) and two additional double bonds (C_7-C_8 , $C_{22}-C_{23}$), while stigmasterol has an ethyl group at C_{24} and an external double linkage at $C_{22}-C_{23}$, in addition to the usual double bond at C_7-C_8 .

Cholesterol (Gr. *chole*, bile + *stereos*, solid) has been known since the eighteenth century as the chief constituent of human gall stones. It is the characteristic sterol of higher animals and it is present in all cells of the animal organism, in largest amounts in the brain and nerve tissue, in the suprarenal glands, and in egg yolk. The solid matter of the human brain contains as much as 17% of the substance. A good grade of cholesterol prepared from the spinal cord of cattle is available

² R. J. Anderson, *J. Am. Chem. Soc.*, **46**, 1450 (1924); Anderson, Nabenhauer and Shriver, *J. Biol. Chem.*, **71**, 849 (1927); Anderson and Shriver, *ibid.*, **71**, 401 (1927); *J. Am. Chem. Soc.*, **48**, 3976 (1926); Anderson, Shriver and Burr, *ibid.*, **48**, 2087 (1926); Nabenhauer and Anderson, *ibid.*, **48**, 2972 (1926).

commercially, but while this material is suitable for most preparative purposes it contains small amounts of closely related substances which cannot be eliminated by repeated crystallization. One of these is the

PROPERTIES OF THE STEROLS

Name	Formula	Double bonds	M.p.	$[\alpha]_D$	Occurrence
Cholesterol	$C_{27}H_{48}O$	1	150°	-38.8°	All animal cells
Dihydrocholesterol	$C_{27}H_{50}O$	0	142°	+28.8°	Companion of cholesterol
Coprosterol	$C_{27}H_{48}O$	0	102°	+23.5°	Feces
Ostreasterol	$C_{28}H_{48}O$	2	143°	-43.9°	Oysters, gastropods
Lanosterol	$C_{28}H_{48}O$	2	141°	+58°	Wool fat
Agnosterol	$C_{28}H_{48}O$	3	162°	+70.6°	Wool fat
Ergosterol	$C_{28}H_{44}O$	3	163°	-133°	Ergot, yeast
γ -Sitosterol	$C_{27}H_{48}O$	1	146°	-42.4°	Fats of higher plants
Stigmasterol	$C_{27}H_{46}O$	2	170°	-45°	Calabar bean, soy bean
Cincho	$C_{21}H_{36}O$	1	140°	-34°	Cinchona bark
Fucosterol	$C_{26}H_{44}O$	2	121°	-38.4°	Algae
Zyino-sterol	$C_{27}H_{44}O$	2	110°	+47.3°	Yeast

saturated alcohol dihydrocholesterol, which Schoenheimer³ has found present to the extent of 1-2% in cholesterol from various organs. Ergosterol, or some other highly unsaturated sterol, also appears to be present in slight traces. A purification is conveniently accomplished through the characteristic and sparingly soluble dibromide,⁴ from which the unsaturated alcohol is regenerated by debromination with zinc dust and acetic acid or, more satisfactorily, by means of sodium iodide.⁵

Stereochemistry of the Sterols. Coprosterol (Gr. *kopro*, dung) is a saturated alcohol isomeric with dihydrocholesterol. The difference is of a stereochemical nature and one of fundamental importance in the chemistry of both the sterols and the bile acids. The spatial arrangement of the hydroxyl group at C_3 is not involved, for the isomeric relationship is maintained in the saturated hydrocarbons cholestane and coprostane. Chemical evidence adduced by Windaus⁶ has established with a high degree of probability if not with certainty that these hydrocarbons differ from one another in the nature of the union between rings A and B, as shown in the formulas. In cholestane the configuration of rings A and B corresponds to that of trans decalin (two "chair" cyclohexane rings), while coprostane (also called pseudocholestane) corresponds to cis decalin (two "bed" rings). Ruzicka⁷ has found confirmation in this view in the

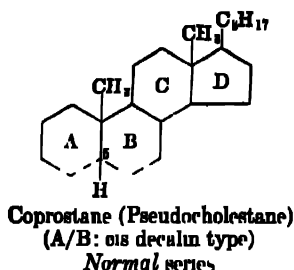
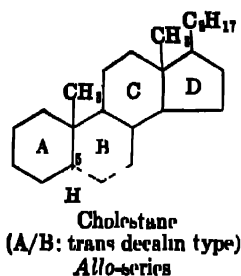
³ Schoenheimer, v. Behring, Hummel and Schudel, *Z. physiol. Chem.*, **192**, 73 (1930); Schoenheimer, v. Behring and Hummel, *ibid.*, **192**, 83 (1930).

⁴ Windaus, *Ber.*, **39**, 516 (1906); Windaus and Hauth, *ibid.*, **39**, 437^a (1906); R. J. Anderson, *J. Biol. Chem.*, **71**, 407 (1927).

⁵ Schoenheimer, *Z. physiol. Chem.*, **192**, 86 (1930); *J. Biol. Chem.*, **110**, 461 (1935).

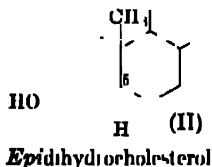
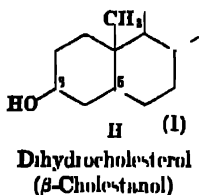
⁶ Windaus, *Lan.*, **447**, 233 (1926).

⁷ Ruzicka, Furter and Thomann, *Helv. Chim. Acta*, **16**, 327 (1933).



definite if slight differences in the densities and the molecular refractions of the two hydrocarbons. It will be observed that the isomerism is dependent specifically upon the configuration at the asymmetric center C₁₀, the hydrogen atom at this point bearing either the *cis* or the *trans* relationship to the methyl group at C₁₃. For reasons which will become apparent on considering the chemistry of the bile acids, cholestane is conventionally classed as a member of the *allo*-series.

Another important type of stereoisomerism has to do with the configuration of the carbon atom (C₃) which in all of the known sterols of natural occurrence is the point of attachment of the lone hydroxyl group. By convention the configuration of cholesterol or of dihydrocholesterol is represented as in I, and that of the epimeric form as in II. In I the

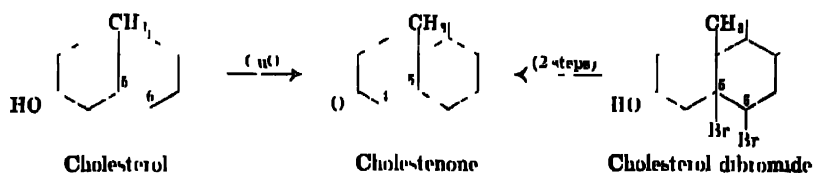


hydroxyl group bears the *trans* relationship to the hydrogen atom at C₁₀, while in II the substituents are *cis*. Dihydrocholesterol is also called β -cholestanol, and substances of this configuration are said to be of the " β -type." All of the natural sterols are β -compounds.

The conversion of cholesterol into substances of both the coprostan series and the *epi*-type was studied most extensively in connection with recent investigations in the field of the sex hormones (Chapter V), but the methods may be described at this point in order to illustrate further the stereochemical relationships. The sole product of the hydrogenation of cholesterol in the presence of platinum catalyst is dihydrocholesterol,⁸ but three important stereoisomers can be prepared from the ketones

⁸ Willstätter and E. W. Mayer, *Ber.*, 41, 2190 (1908)

cholestanone and cholestenone. The saturated ketone is obtained by the oxidation of the readily available dihydrocholesterol (β -cholestanol), while cholestenone, an α , β -unsaturated ketone, can be prepared in good yield from cholesterol by two different methods. Either cholesterol is dehydrogenated directly over copper oxide,⁹ or it is converted into the



dibromide in order to protect the double bond and the dibromide is oxidized and debrominated.¹⁰ In both the high- and low-temperature reactions the double bond migrates from the 5,6-position to a position of conjugation at C₄-C.

The methods of converting these ketones into the four stereoisomeric alcohols are indicated in the chart on page 116. When cholestanone is hydrogenated in a neutral medium, or when it is reduced with sodium and amyl alcohol, the chief product is dihydrocholesterol,¹¹ but Vavon and Jakubowicz¹² discovered that in the presence of a small amount of hydrochloric acid the catalytic hydrogenation takes a different stereochemical course and gives *epidihydrocholesterol* as almost the exclusive product. Previously *epidihydrocholesterol* had been obtained in small amounts by the epimerization of dihydrocholesterol with sodium ethylate, but the reaction reaches a point of equilibrium when only about 10% of the material has been isomerized.¹³ No good route to compounds of the coprostane series was available until Grasshof¹⁴ discovered that coprostanone can be obtained in good yield by the partial hydrogenation of cholestenone. The C₄-C₅ double bond theoretically can open in two ways, with the establishment of either of the alternate configurations at C₅, and the course of the reduction evidently is dependent upon the conditions, for Diels and Abderhalden¹⁵ obtained dihydrocholesterol from cholestenone by reduction with sodium and amyl alcohol. With coprostanone readily available, Grasshof¹⁵ found that coprosterol can be obtained by the hydrogenation of the ketone in an acidic medium (glacial acetic acid), while Ruzicka and co-workers¹⁶ found that in ethereal solu-

⁹ Diels and Abderhalden, *Ber.*, **37**, 3000 (1904), Diels, Gailler and Kurling, *Ann.*, **459**, 21 (1927).

¹⁰ Windaus, *Ber.*, **39**, 518 (1906), Schoenheimer, *J. Biol. Chem.*, **110**, 461 (1935).

¹¹ Diels and Abderhalden, *Ber.*, **39**, 3361 (1906).

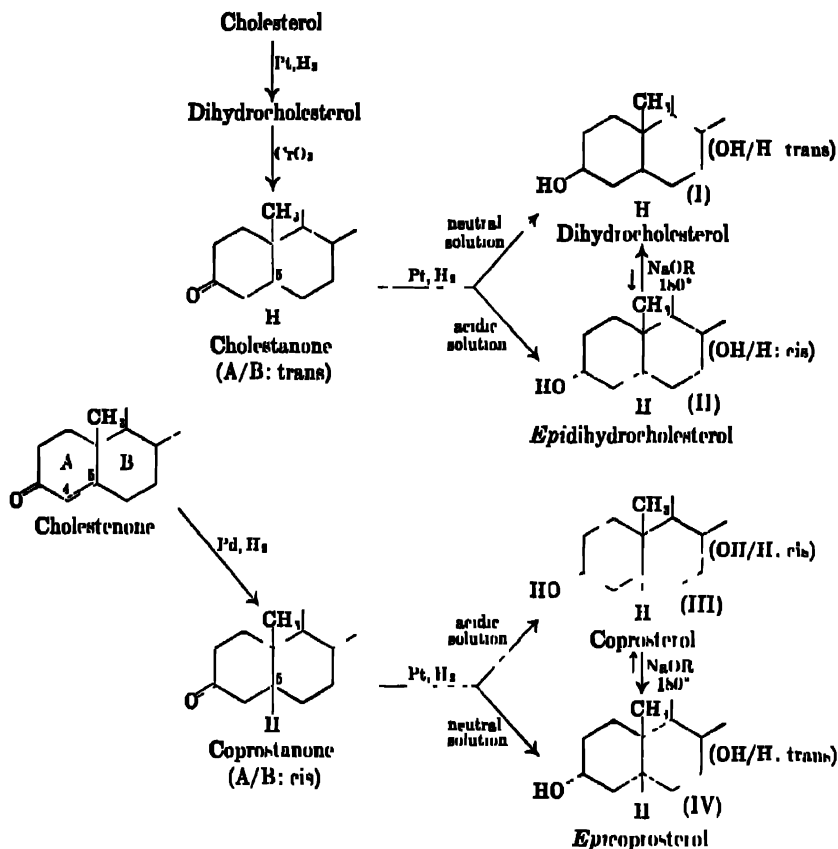
¹² Vavon and Jakubowicz, *Bull. soc. chim.*, [4], **53**, 551 (1933).

¹³ Windaus and Uhlig, *Ber.*, **47**, 2394 (1914).

¹⁴ Grasshof, *Z. physiol. Chem.*, **223**, 249 (1935).

¹⁵ *Idem*, *ibid.*, **225**, 197 (1935).

¹⁶ Ruzicka, Brüniger, Eichenberger and J. Meyer, *Helv. Chim. Acta*, **17**, 1407 (1934).



tion the chief product of hydrogenation is *epicoprosterol*. The interconversion of the epimers had been observed earlier by Windaus.¹⁷

The convention regarding the stereochemical arrangement of the hydroxyl group with respect to the hydrogen atom at C_7 (OH/H:trans, in the case of dihydrocholesterol) is due to Ruzicka¹⁶ and is based upon the Auwers-Skita rule that catalytic hydrogenation in an acidic medium usually gives cis forms, whereas trans compounds are produced in a neutral solution. The configurations indicated in formulas I-IV are all consistent with the rule, and if it is the arrangement of the hydrogen atom at C_7 which determines the mode of addition to the carbonyl group the configurations are probably correct. The formation of preponderant amounts of the trans compounds I and IV on rearrangement with sodium ethylate is also consistent with this definition of the cis-trans relationship. It is uncertain, however, whether the spatial arrangement of the

¹⁷ Windaus and Uibrig, *Ber.*, **48**, 857 (1915); Windaus, *ibid.*, **49**, 1724 (1916).

hydrogen atom at C₅ or that of the bond extending from C₅ into ring B should be considered in applying the Auwers-Skita rule, and it is possible that the configuration at C₅ is the opposite of that conventionally attributed to each of the compounds I-IV. This reservation was clearly recognized by Ruzicka.

Information as to the stereochemical arrangement of the remainder of the cholesterol molecule is still incomplete, although there are indications of a trans linkage between rings B and C and between rings C and D. The arrangement, however, appears to be the same in all of the sterols and related natural products, and there has been little occasion to consider isomerism arising from asymmetric centers other than C₅ and C₈.

Color Reactions. When treated with strong acids under dehydrating conditions, the colorless sterols often give rise to beautiful and varied displays of color. Often there is a period of induction followed by the gradual development of a succession of colors. These phenomena form the basis of a number of color tests, the more useful of which may be listed as follows:

*The Liebermann*¹⁸ or *Liebermann-Burchard*¹⁹ reaction. A solution of a small crystal of the sterol in cold acetic anhydride is treated with a few drops of concentrated sulfuric acid (Liebermann), or the sterol is dissolved in chloroform and treated with acetic anhydride and sulfuric acid.

*The Salkowski reaction.*²⁰ A solution of the material in chloroform is shaken with concentrated sulfuric acid (colors in both layers).

*The Lifschutz reaction.*²¹ Small quantities of the sterol and of perbenzoic acid are heated in glacial acetic acid solution and sulfuric acid is added.

*The Tschugajeff reaction.*²² A glacial acetic acid solution of the sterol is treated with zinc chloride and acetyl chloride and boiled.

*The Rosenheim reaction.*²³ The substance to be tested is dissolved in chloroform and a few drops of an aqueous solution of trichloroacetic acid are added.

Further elaborations of some of these tests are described by Whithy²⁴ and by Schoenheimer.²⁵ The latter author²⁶ compared different methods for the colorimetric estimation of small quantities of (unsaturated) sterols

¹⁸ Liebermann, *Ber.*, 18, 1808 (1885)

¹⁹ Burchard, Inaugural Dissertation, Rostock (1899), see *Chem. Zentr.*, 1, 25 (1899)

²⁰ Salkowski, *Z. physiol. Chem.*, 57, 628 (1908)

²¹ Lifschutz, *Ber.*, 41, 252 (1908)

²² Tschugajeff, *Chem. Ztg.*, 24, 542 (1900)

²³ Rosenheim, *Biochem. J.*, 23, 47 (1929)

²⁴ Whithy, *ibid.*, 17, 5 (1923)

²⁵ Schoenheimer, Dam and v. Goltberg, *J. Biol. Chem.*, 110, 659 (1935).

²⁶ Schoenheimer, *Z. physiol. Chem.*, 192, 77 (1930).

and found them for the most part less reliable than the pyridine sulfate dibromide method (Dam²⁷) or the bromine-methanol method (Kaufmann²⁸).

The color reactions distinguish sharply the unsaturated from the saturated sterols, for the tests are negative with dihydrocholesterol, coprosterol, etc. The colored substances probably are halochromic salts formed by the attachment of the acid or the metal salt to an unsaturated center of the molecule. The acetic anhydride or acetyl chloride may enter into the formation of the coördinate complex or it may react chemically with the unsaturated compound. It also appears possible that dehydration to a more highly unsaturated substance may occur at some stages of the reactions. The Rosenheim reaction appears to be characteristic of the triply unsaturated ergosterol and of allocholesterol,²⁹ a labile isomer of cholesterol having the double bond at the 4,5-position. It should be noted that the color reactions are not specific for the sterols but that many other polynuclear hydroaromatic compounds which are unsaturated or potentially unsaturated behave in a comparable manner.

The color tests are very useful for the detection of traces of unsaturated sterols in the presence of the saturated compounds, as, for example, in determining the purity of a product of hydrogenation. Anderson and Nabenhauer³⁰ employed a modified Liebermann-Burchard reaction in a procedure for the purification of a saturated sterol. A solution of the crude material in carbon tetrachloride is shaken with acetic anhydride and sulfuric acid, and water is added until the colored compound containing the unsaturated sterol separates as a thick layer from the clarified solution.

Precipitation with Digitonin. Many applications have been made of the important discovery by Windaus³¹ that cholesterol forms a remarkably stable and sparingly soluble molecular compound with digitonin, a glycosidic saponin of the composition $C_{56}H_{92}O_{24}$ (page 321). The compound, called cholesterol digitonide, is made up of one molecule each of cholesterol and digitonin. The substance has no definite melting point, but it separates from solutions in a characteristically crystalline form. The solubility is so slight (0.014 g. in 100 cc. of 95% alcohol at 18°) that the compound is easily freed from foreign materials. Dissociation into the components occurs in alcoholic solution to a definite, but very slight, extent. The precipitation of cholesterol from an alcoholic solution by digitonin affords a sensitive qualitative test for the presence of the sterol,

²⁷ Dam, *Biochem Z*, 152, 101 (1924).

²⁸ H. P. Kaufmann, *Z. Unters. Lebensmittel*, 51, 3 (1926).

²⁹ Windaus, *Ann*, 453, 101 (1927).

³⁰ R. J. Anderson and Nabenhauer, *J. Am. Chem. Soc.*, 46, 1957 (1924).

³¹ Windaus, *Ber.*, 42, 239 (1909).

a reaction being observable at a dilution of 1:10,000. The reaction also serves as the basis for the quantitative determination of cholesterol, the precipitated digitonide being collected and weighed. The original method of Windaus³² has been adapted to various purposes, and is used extensively for the microdetermination of cholesterol in biological material.³³ Use is also made of the precipitation of cholesterol and other sterols by digitonin in separating sterols from mixtures. Special methods are required for the recovery of the sterols and of the costly digitonin, for the molecular compounds are so stable that they are not decomposed, for example, by continued extraction with ether. Boiling with xylene for several hours serves to extract the sterol portion, but certain sterols are far too sensitive for such drastic treatment. One method of cleavage depends upon the fact that the acetyl derivatives of the sterols usually do not combine with digitonin: after treatment of cholesterol digitonide with acetic anhydride the cholesteryl acetate can be extracted with ether (Windaus³²). An improved method consists in dissolving the molecular compound in cold pyridine, in which dissociation is apparently complete, and precipitating the digitonin with ether.³⁴ The sterol remains in solution. This method of cleavage is of value in isolating such substances as allocholesterol, which easily loses a molecule of water.

The presence in cholesterol of the free hydroxyl group appears to be essential to the formation of the molecular compounds, since cholesteryl acetate (or the *i*-butyrate or palmitate) is not precipitated by digitonin, and the same is true of the C₃-halogen compounds and ketones.³⁵ The configuration of the carbon atom carrying the hydroxyl group must, however, correspond to that of cholesterol. Dihydrocholesterol combines with digitonin but *epi*dihydrocholesterol is not precipitated by the reagent. On the other hand, the manner of linkage between rings A and B is not a factor of importance, for coprosterol (but not *epi*coprosterol) forms a molecular compound as well as dihydrocholesterol. Digitonin also combines with the phytosterols stigmasterol, sitosterol, ergosterol, and fucosterol, and it is known from degradations and interconversions that all of these compounds are of the β -type corresponding in the configuration at C, to dihydrocholesterol. No exceptions have been discovered to the rule that the β -compounds are precipitated with digitonin while the *epi*-compounds are not, and Fernholz³⁶ has shown that the relationship is maintained among degradation products in which the sterol side chain has

³² Windaus, *Z physiol Chem*, **65**, 110 (1910)

³³ Thaysen, *Biochem Z*, **62**, 90 (1914) Frey, *ibid*, **104**, 52 (1920) Szent Gyorgy, *ibid*, **136**, 107 (1923), Hanf, "Mikromethoden zur Blutuntersuchung" 3d Ed (1923)

³⁴ Schonheimer and Dan, *Z physiol Chem*, **215**, 50 (1931)

³⁵ Recent work of Butenandt (page 240) indicates that there are exceptions to this rule. See also Butenandt, Tscherning and Haasch, *Ber*, **68**, 3017 (1935)

³⁶ Fernholz, *Z physiol Chem*, **232**, 97 (1935)

been shortened or even replaced by a ketonic group (sex hormones). The importance of the position of the hydroxyl group has not been thoroughly investigated, but it is known that 4-cholestanol is not precipitated by digitonin.

The remarkable space specificity of the digitonin reaction is of great value in stereochemical studies in the sterol series and the reagent can be used for diagnostic purposes with a considerable degree of assurance. It is also very useful in preparative work for the removal from indifferent material of traces of substances capable of combining with the reagent. For example in the preparation of *epicoprosterol* by the hydrogenation of coprostanone in ethereal solution a completely pure product is easily obtained by precipitating traces of coprosterol with digitonin. For the separation of saturated sterols (of the β -type) from cholesterol or other unsaturated sterols, Schoenheimer²⁸ developed a method based on the observation that the sterol bromides are not precipitable with digitonin. The mixture is treated carefully with bromine to convert the cholesterol present into the dibromide, and digitonin then precipitates only the saturated sterols.

The Origin and Function of the Sterols.³⁷ It has been demonstrated experimentally that the lower plants can synthesize sterols from simple substances such as the sugars, and in the case of the higher plants it has been shown that the formation of sterols can occur at any stage of development. Until a comparatively recent date it was generally believed that the plants alone are capable of effecting the synthesis of the complicated sterol structures. Since cholesterol, the specific sterol of man and higher animals, does not occur in plants even in traces, it was assumed that the animal body assimilates phytosterols from the diet and transforms these substances into cholesterol. From a knowledge of the structures it is clear that such a process would have to involve the loss of methyl or ethyl groups from the phytosterol side chains, but there is evidence more important than that based on the argument of improbability to show that the assumption is not valid and that the animal organism is capable of producing its own sterols.

The question has been investigated in cholesterol-balance studies of metabolism, and under certain special conditions cases have been observed where the amount of cholesterol excreted is greater than that consumed. This is an indication that the animal has the power to produce cholesterol, a conclusion which has been confirmed by feeding experiments with sterol-deficient diets. Of course the demonstration that under certain conditions cholesterol can be formed in the body is no proof that all of the cholesterol normally present is due to a synthesis in the organism, and it is necessary

³⁷ See C. E. Bills, "Physiology of the Sterols, including Vitamin D," *Physiol. Rev.*, 15, 1 (1935)

to consider the possibility that the major part comes from vegetable foods. In order for plant sterols to be converted into cholesterol in the animal body they would have to be capable of absorption,³⁸ and Schoenheimer conducted extensive investigations of the absorbability of various sterols with the idea that this might provide a definite answer to the question.³⁹ The work was suggested by observations in connection with experiments on the production of atherosclerosis in rabbits. These animals are accustomed to a purely vegetable diet, that is, to foods containing phytosterols but no cholesterol. When cholesterol is added to the diet the rabbits develop morphologically visible deposits of cholesterol in various organs and the general change strongly resembles human atherosclerosis. No such deposits are formed under normal dietary conditions, and yet if the phytosterols present in the ordinary feed of rabbits were all converted into cholesterol the amount of this substance would be ten times as high as the dose required to produce atherosclerosis. In investigating the matter further, Schoenheimer fed huge doses of pure sitosterol to rabbits and found that, in contrast to the results with cholesterol, the animals remained healthy and showed no signs of pathological changes. The animals contained no more cholesterol than the normal ones, and quantitative collection and analysis of the feces showed that the sitosterol fed is completely excreted.

These and other studies proved conclusively that the phytosterols are not capable of being absorbed in the animal organism.⁴⁰ A particularly crucial test was possible in the case of ergosterol, for very minute traces of this substance can be detected with the aid of the absorption spectrum and by a biological method depending upon the development of anti-rachitic activity following irradiation. The amount of ergosterol found in body sterols following feeding experiments was no higher than normal, but irradiated ergosterol, on the other hand, was found to be easily absorbed. The animal organism evidently has remarkable powers of selection. Absorbability is highly dependent upon chemical constitution for, in contrast to cholesterol, such closely related substances as dihydrocholesterol, coprosterol, and allocholesterol are not appreciably absorbed through the intestinal wall but pass directly into the feces.⁴¹

The situation is somewhat different with regard to the lower animals, for Bergmann⁴² discovered that the unsaponifiable matter from oysters and other bivalves contains in place of cholesterol a C₂₀-sterol, ostreas-

³⁸ Physiological absorption is defined as the passage of digested food through the alimentary canal into the blood or lymph for transference to various parts of the body.

³⁹ For a review, with references, see Schoenheimer, *Science*, 74, 579 (1931).

⁴⁰ Dam and Starup, *Biochem. Z.*, 278, 312 (1933), found that intravenously injected phytosterol is eliminated by the dog or rabbit chiefly in the feces.

⁴¹ Schoenheimer, Dam and v. Gotthberg, *J. Biol. Chem.*, 110, 667 (1935).

⁴² W. Bergmann, *ibid.*, 104, 317, 553 (1931).

terol. It is a doubly unsaturated compound having two carbon atoms more than cholesterol, and the close relationship of this zoosterol to the common phytosterols is shown by the fact that the substance is an isomer of stigmasterol and yields sitostanol on hydrogenation. Bergmann's observation, "it seems possible that certain molluscs are unable to synthesize cholesterol but that they use directly or in dehydrogenated form the phytosterols of their food, which consists mainly of algae or diatoms," finds an interesting corollary in Heilbron's⁴³ isolation of fucoesterol from algae. This phytosterol is an isomer of ostreasterol and can be converted into stigmastanol.

Although the evidence is conclusive that the cholesterol present in higher animals is synthesized in the animal organism, the site and the mechanism of cholesterol formation are unknown. Whether it comes from fats or other primary materials still remains to be determined. Also in large part unsolved is the problem of defining the functions of the sterol in the organism. From considerations which will be outlined in later sections it seems highly probable that cholesterol is the parent substance from which the organism produces the important acids of the bile and the sex hormones, although the relation has not been established physiologically. Possibly the antirachitic vitamin D comes from the same source or from a companion substance. However, the amount of cholesterol present in the body appears out of proportion with that which would be adequate to meet these requirements, and the distribution of the substance is suggestive of other functions. From the abundance of cholesterol in the brain and nerves it has been suggested that the substance may be a protective agent for nervous matter. The biological function may be in part associated with the ability of cholesterol to precipitate hemolytic saponins, and the sterol may play a part in controlling cell permeability. None of the various functions suggested has been securely established and the problem awaits further investigation.

Studies of sterol metabolism have shown that the body eliminates little cholesterol as such. Undoubtedly some is completely consumed within the organism, for it has been demonstrated⁴⁴ in experiments with mice that on diets rich in cholesterol the animals can destroy several times the normal body content of cholesterol. Some of the material probably is transformed into bile acids and sex hormones, and a small part is transformed by hydrogenation in the body tissues into dihydrocholesterol, which finds its way to the feces through the intestinal wall.⁴⁵ Coprosterol, unlike its stereoisomeride, is not found in the tissues but it occurs in

⁴³ Heilbron, Phipps and Wright, *J. Chem. Soc.*, 1372 (1934), Coffey, Heilbron, Spring and Wright *ibid.*, 1205 (1935)

⁴⁴ Schoenheimer and Breuer, *J. Biol. Chem.*, 103, 439 (1933)

⁴⁵ Schoenheimer and v. Bering, *Z. physiol. Chem.*, 192, 102 (1930)

much larger amounts in the feces. It has been generally assumed that coprosterol is formed from cholesterol by the action of reducing bacteria in the intestines, but Schoenheimer⁴⁶ has recently questioned this assumption. Under all known conditions of hydrogenation in the laboratory cholesterol yields only dihydrocholesterol, and the reduction which occurs to a slight extent in the tissues gives the same isomer uncontaminated with coprosterol. Schoenheimer has suggested that coprosterol may arise not as a product of direct hydrogenation but through one or more intermediary products such as cholostenone or coprostanone. The ketones may be formed through an oxidation mechanism and converted into coprosterol by reducing bacteria. Preliminary support for this view has been obtained in highly ingenious feeding experiments using deuterium as an indicator to establish the identity of products isolated from the feces. Employing coprostanone-4,5d₂, Schoenheimer was able to demonstrate the biological conversion of coprostanone into coprosterol.

The Bile Acids. Human bile is a golden brown liquid having an alkaline reaction (pH 7.8-8.6) and containing inorganic salts, bile salts (sodium salts of conjugated bile acids), and small amounts of cholesterol, lecithin, and bile pigments. The principal pigment is bilirubin, an oxidation product of hemin. Bile is produced in the liver, stored in the gall bladder, and secreted in small amounts into the intestines, and its chief function is to promote the resorption of fats and of cholesterol in the intestinal tract. The bile salts, which are the chief constituents of the solid matter of bile, have the specific power of keeping water-insoluble substances in solution or dispersion. Whether they function entirely as emulsifying agents or form water-soluble molecular compounds with the fats (page 131), the bile salts enable the lipoids to become absorbed on the intestinal mucosa. By enhancing the action of hydrolytic enzymes on the absorbed fats, the bile salts further promote the diffusion of the substances through the membrane, while the salts themselves are returned to the solution.

After submitting the bile of an animal to alkaline hydrolysis, an acidic fraction may be obtained which is composed of a mixture of bile acids. The material from ox bile consists chiefly of cholic acid and desoxycholic acid, with smaller amounts of chenodesoxycholic acid, lithocholic acid, and other related substances. The structures of the four principal bile acids from this source are indicated in the formulas on page 124, and it will be seen that they all have the same carbon skeleton and that they differ only in the number and positions of the secondary alcoholic groups.

⁴⁶ Schoenheimer, Rittenberg and Guff, *J. Biol. Chem.*, 111, 193 (1935). See also Rosenheim and Webster, *Nature*, 136, 474 (1935).

Glyco- and tauro-desoxycholic acid also have been isolated and the other bile acids are known to occur in conjugation with the same amino acids. The glyco-acids are somewhat more abundant constituents of human bile and ox bile than the tauro-compounds, but there are variations from species to species. Hog bile contains principally glyco-acids, while codfish bile is particularly rich in tauro-bile acids. The conjugated acids are somewhat more strongly acidic than the free acids. The isolation of the conjugated acids from bile is a troublesome and uncertain procedure, and the free acids are much more readily available. The synthetic conjugation of the components was accomplished at an early date,⁴⁷ but a practical method has become available only recently. Cortese and Bauman⁴⁸ protected the hydroxyl groups of cholic acid with formyl groups, prepared the acid chloride, and coupled this with glycine in alkaline solution, the formyl groups being hydrolyzed in the latter process.

Cholic acid is the most abundant of the acids obtainable from ox bile and the isolation is not a difficult matter. Desoxycholic acid can be prepared fairly easily from the same source,⁴⁹ but the isolation of the other acids of ox bile is a very difficult matter. According to Wieland and Weyland⁵⁰ 100 kg. of ox bile yields 5-6 kg. of cholic acid, 600-800 g. of desoxycholic acid, and about 2 g. of lithocholic acid. Chenodesoxycholic acid appears to be present in even smaller amounts than lithocholic acid. Lithocholic acid (Gr. *lithos*, stone) was obtained for the first time not from bile but from gall stones. H. Fischer⁵¹ discovered this rare acid in 1911 in an investigation which had as the primary object the study of the bile pigment bilirubin, and which initiated the brilliant series of investigations of the blood pigments from which bilirubin arises as an oxidation product. The bilirubin was extracted from the gall stones and lithocholic acid appeared as an incidental product in the course of the separations.

Investigations of the constituents of the bile of various animals⁵² have furnished many interesting and useful results. The hydroxycholic acids thus far isolated are listed in the table, which includes references regarding the less common of the substances. Hyodesoxycholic acid (Gr. *hyo-*, swine), which is readily obtainable from hog bile, has been

⁴⁷ Bondi and E. Müller, *Z. physiol. Chem.*, **47**, 499 (1906); Wieland and Stender, *ibid.*, **106**, 151 (1919)

⁴⁸ Cortese and Bauman, *J. Am. Chem. Soc.*, **57**, 1343 (1935)

⁴⁹ Simplified procedures for the isolation of cholic acid and desoxycholic acid are given by Gattermann-Wieland, "Laboratory Methods of Organic Chemistry," pp. 394-402, the Macmillan Company, New York, 1932

⁵⁰ Wieland and Weyland, *Z. physiol. Chem.*, **110**, 123 (1920).

⁵¹ H. Fischer, *ibid.*, **73**, 204 (1911)

⁵² For a summary of the literature see N. Okamura and T. Okamura, *ibid.*, **153**, 11 (1930)

BILE ACIDS

Name	Formula	Positions of hydroxyl groups	M p.	$[\alpha]_D$	Sources
Cholic acid	$C_{26}H_{48}O_6$	3,7,12	195°	+ 37°	Man, ox, goat, sheep antelope
β -Phocarcholic acid ⁵¹	$C_{26}H_{48}O_6$	3,7,23	222°	+ 28°	Walrus, seal
Nutriacholic acid ⁵⁴	$C_{26}H_{48}O_6$		198°		Swamp beaver
Desoxycholic acid	$C_{24}H_{40}O_5$	3,12	176°	+ 55°	Man, ox, goat, sheep, deer, antelope
Chenodesoxycholic acid ⁵⁵	$C_{24}H_{40}O_5$	3,7	110°	+ 11°	Man, ox, goose, hen
Hyodesoxycholic acid ⁵⁶	$C_{26}H_{48}O_5$	3,6	197°		Hog, hippopotamus
Ursodesoxycholic acid ⁵⁷	$C_{26}H_{48}O_5$	3,(?)	198°		Bear
Bufodesoxycholic acid ⁵⁸	$C_{26}H_{48}O_5$				Toad
Lithocholic acid ⁵⁹	$C_{21}H_{40}O_4$	3	186°	+ 32°	Man, ox

of considerable importance in the studies of structure. Windaus⁵⁶ found that hyodesoxycholic acid is a 3,6-dihydroxy compound and consequently that only one of the substituents corresponds in position to the hydroxyl groups of cholic acid. This is also true of the ursodesoxycholic acid (*L. ursus*, bear) described by Kapiro.⁵⁷ The discovery of 3,7-dihydroxy-cholanic acid was announced simultaneously by Wieland⁵⁸ and by Windaus⁵⁵ in 1924. Wieland isolated this isomer of desoxycholic acid from human bile and called it anthropodesoxycholic acid (Gr. *anthropo*, man), while Windaus discovered the substance in the bile of the goose and named it chenodesoxycholic acid (Gr. *cheno*, goose). Although the two investigators have reached no agreement as to the name, it would seem that Windaus' designation may be given the greater weight because the prefix originated with Heintz and Wislicenus, who apparently had obtained the acid in question from goose bile in 1859 but had failed to achieve a purification sufficient to reveal the true formula. Bile from cadavers contains nearly as much chenodesoxycholic acid as of the isomeric 3,12-acid, while the amount present in ox bile is extremely small.⁶⁰

⁵¹ Structure Windaus and van Schoor, *Z. physiol. Chem.*, **173**, 312 (1924)

⁵² Isolation Brühl and Benedict, *ibid.*, **220**, 106 (1923)

⁵³ Isolation. Windaus, Bohne and Schwarzkopf, *ibid.*, **140**, 177 (1921) Wieland and Revery, *ibid.*, **140**, 146 (1924).

⁵⁴ Structure Windaus and Bohne, *Ann.*, **433**, 275 (1923) Windaus, *ibid.*, **447**, 233 (1923), *Z. anorg. Chem.*, **36**, 309 (1923) An improved procedure for the isolation of the acid is described by Wieland and Gümlich, *Z. physiol. Chem.*, **215**, 18 (1933)

⁵⁵ Structure Kapiro Kapiro, *ibid.*, **185**, 151 (1924), **197**, 206 (1931)

⁵⁶ Isolation and characterization of the non-crystalline product T. Okamura, see *Chem. Zinb.*, **1**, 2624 (1928), **1**, 1113 (1929), **1**, 1310 (1930)

⁵⁷ Isolation H. Fischer, *Z. physiol. Chem.*, **73**, 204 (1911) Characterization Wieland and Weyland, *ibid.*, **110**, 123 (1930)

⁵⁸ Wieland and R. Jacobi, *ibid.*, **148**, 232 (1925)

Nearly all of the bile acids have been submitted to dehydration followed by hydrogenation, or converted by other methods into the hydroxyl-free acids. As stated above cholic acid, desoxy-, chenodesoxy-, and lithocholic acids all yield the same substance, cholanic acid, but this is not true of any of the other compounds investigated. Although hyodesoxycholic acid belongs to the stereochemical series of cholanic acid (and coprostane), a stereochemical inversion occurs in the course of the degradation for the saturated acid has a different configuration and is called *allocholanic acid*. The substance has been shown to belong to the same series as cholestane and dihydrocholesterol (rings A/B: trans decalin type). The configuration of the other cholanic acids (see table) is unknown.

THE CHOLANIC ACIDS

	Mp	$[\alpha]_D$	Source
Cholanic acid ⁶¹	168°	+ 21.7°	Coprostane, cholic acid, desoxy-, chenodesoxy-, and lithocholic acid
Allocholanic acid ⁶²	170°	+ 22.5°	Cholestane, hyodesoxycholic acid, scallardin A (p 295)
Ursocolanic acid ⁶³	153°	- 34.1°	Ursodesoxycholic acid
Buforholanic acid ⁶⁴	230°	- 20.3°	Bufodesoxycholic acid
Isobuforholanic acid ⁶⁵	179°	+ 50.5°	Bufotilin (p 306)

One objective in view in the continued search for new bile acids and for companion substances is the establishment of the physiological relationship of the bile acids to other products of the animal body. A knowledge of the structures of intermediate products of metabolism might reveal the origin and the fate of the acids of the bile, and some advances in this direction already have been made. From the residues of the commercial preparation of cholic and desoxycholic acids from ox bile Wieland and Kishi⁶⁶ isolated two new acids, the first of which was shown to be 3-hydroxy-12-ketocholanic acid. An isomeric substance, 3-hydroxy-6-keto-*allocholanic acid* was isolated by Fernholz⁶⁷ from hog bile. Hydroxyketo acids of this type may be the natural precursors of the polyhydroxy acids of the bile. That the reduction of keto acids can occur in the body has been demonstrated by Yamasaki and Kyogoku,⁶⁸ who injected 3,7,12-triketocholanic acid subcutaneously into toads and investigated the mate-

⁶¹ Wieland and Weil, *Z. physiol. Chem.*, **80**, 257 (1912); Wieland and Boersch, *ibid.*, **106**, 193 (1919); Windaus and Neukirch, *Ber.*, **52**, 1918 (1919).

⁶² Windaus and Neukirch, loc. cit. Windaus and Bolme, *Ann.*, **433**, 281 (1923).

⁶³ Shoda, see *Chem. Zentr.*, **2**, 679 (1928); Kashi, *Z. physiol. Chem.*, **185**, 151 (1929).

⁶⁴ T. Okumura, *J. Biochem. (Jap.)*, **10**, 5 (1925), **11**, 103 (1926).

⁶⁵ Wieland, Hesse and U. Moyer, *Ann.*, **493**, 272 (1932).

⁶⁶ Wieland and Kishi, *Z. physiol. Chem.*, **214**, 17 (1933).

⁶⁷ Fernholz, *ibid.*, **214**, 47 (1933).

⁶⁸ Yamasaki and Kyogoku, *ibid.*, **233**, 29 (1935); **235**, 43 (1935).

ring is indicated by the further oxidation of the above 27-carbon acid to a 24-carbon triketocholanic acid. The oxide ring must be linked either at C_{24} - C_{25} as indicated in the formula or at C_{25} - C_{27} . The triketo acid differs from 3,7,12-triketocholanic acid obtained from cholic acid, but it yields the known 7,12-diketocholanic acid on Clemmensen reduction. This fixes the ring system and the location of two of the secondary hydroxyl groups (at C_7 and C_{12}). The third such group cannot occupy position C_4 and, since the triketo acid appears capable of ready enolization in alkaline solution, the most probable point of attachment is at C_4 (conjugated dienol). Although there are still two points of uncertainty regarding the structure, it is clear that scymnol has the same number and arrangement of carbon atoms as cholesterol and coprosterol. Since it apparently lacks the characteristic hydroxyl group at C_3 , scymnol can hardly be a direct oxidation product of either of these substances, but it may well come from a related sterol or from a common parent substance.

The Choleic Acids. Cholic acid is a colorless, crystalline solid of high melting point and slight solubility in water. It separates from solutions in alcohol or in aqueous acetic acid with one molecule of alcohol or water of crystallization, and the solvent is held very tenaciously and can be removed completely only after prolonged heating at reduced pressure.

With desoxycholic acid the property of forming stable molecular compounds is magnified to a high degree. The unique character of the 3,12-dihydroxy acid was discovered by Wieland and Sorge¹³ in an important investigation which originated in an interesting manner. A substance known as "choleic acid" had been isolated from bile many years earlier and it was commonly regarded as a true bile acid, probably isomeric with desoxycholic acid. Wieland and Sorge subjected this substance to dehydration by vacuum distillation in the expectation of obtaining an unsaturated acid similar to that resulting from the dehydration of desoxycholic acid. The substance, however, was not an isomer of the choladienic acid but identical with it. There was also formed a small amount of a fatty acid (palmitic or stearic acid), and it was shown that this does not arise through a pyrolytic rupture of the original ring structure, but that it is present in the "choleic acid" in molecular combination with desoxycholic acid. The supposed bile acid is a coordinative compound containing one molecule of the fatty acid and no less than eight molecules of desoxycholic acid. The complex dissolves in alkali without change and the stearic acid is so firmly bound that it can be split off only with difficulty, as by transforming the bile acid into products of dehydration or oxidation. A substance identical with the "choleic acid" crystallizes from an alcoholic

¹³ Wieland and Sorge, *Z. physiol. Chem.*, **97**, 1 (1916)

solution of desoxycholic acid on the addition of stearic acid. The melting point is fairly sharp (186°) and appreciably higher than that of desoxycholic acid. So small is the proportion of the fatty acid that its presence is not easily apparent from the analytical figures.

Following this discovery, Wieland and Sorge and later workers found that desoxycholic acid forms remarkably stable molecular compounds not only with the higher fatty acids but with simple acids, esters, alcohols, ethers, and phenols. Stable additive compounds are formed with cholesterol and with certain aromatic hydrocarbons and certain alkaloids. These unique substances, which are known in a wide variety of types, are now referred to as the choleic acids. Compounds which combine with desoxycholic acid have been called the "acholic" constituents of the choleic acids.⁷⁴

From a systematic study of various series of choleic acids from fatty acids and esters, Rheinboldt⁷⁵ concluded that the ability to combine with desoxycholic acid is dependent upon the size and character of the hydrocarbon part of the acid or ester. In the series of monobasic fatty acids, formic acid alone fails to form a compound. The other acids combine with the bile acid in varying but definite molecular proportions to produce well-defined, crystalline choleic acids. That it is the hydrocarbon residue and not the acid group which is essential is clearly indicated by the ability of desoxycholic acid to combine with normal paraffin hydrocarbons (hexadecane, dodecane).⁷⁶ The choleic acid of acetic acid contains the components in equimolecular proportion, while propionic acid forms a 1:3 compound with desoxycholic acid. The normal acids having 3-7 carbon atoms in the hydrocarbon radical form 1:4 compounds, those having 8-13 carbon atoms combine with 6 molecules of the bile acid, and with the higher acids the ratio is 1:8. Rheinboldt noted that the number of molecules of desoxycholic acid found in combination with the acholic constituent conforms to the coordination principle and may be regarded as a coordination number: 1,2,3,4,6,8. As with other coordination compounds, the space requirements apparently are not satisfied by a cluster of 5 or 7 surrounding molecules. Branching the chain of the acids tends to reduce the coordination numbers, and with such compounds the coordination number is that of the longest straight chain in the molecule.⁷⁷ Hexamethylthane forms no choleic acid in alcoholic solution.⁷⁸

The choleic acids differ greatly in their solubility in organic solvents and in the degree of association of the components in the solutions. Ethyl

⁷⁴ Holotka and Goldberg, *Biochem. J.*, **26**, 555 (1932).

⁷⁵ Rheinboldt, *Ann.*, **451**, 256 (1926); *Z. physiol. Chem.*, **180**, 180 (1929); Rheinboldt, O. König and Otten, *Ann.*, **473**, 240 (1929).

⁷⁶ Finner and Newman, *J. Am. Chem. Soc.*, **57**, 1603 (1935).

⁷⁷ Sobotka and Goldberg, *Biochem. J.*, **26**, 566 (1932); Chargaff and G. Abel, *ibid.*, **28**, 1901 (1934).

alcohol is commonly used for crystallizing desoxycholic acid because it forms a rather labile complex from which the acid can be recovered by prolonged drying in vacuum. Xylene displaces alcohol from the complex even in an alcoholic solution and xylene-choleic acid separates in a crystalline condition. By crystallization from glacial acetic acid the xylene can be displaced by this reagent. Precipitation of the ether complex affords a convenient method of purifying the bile acid.⁷⁴ Naphthalene and some of the higher aromatic hydrocarbons form choleic acids in alcoholic solution, for example acenaphthene, phenanthrene, and methylcholanthrene form crystalline complexes of coordination numbers 2, 3, and 4, respectively.⁷⁵ In each case the melting point is higher than that of either component. Other hydrocarbons (e. g. anthracene, chrysene, 1,2-benzpyrene) crystallize in the free state from alcoholic solutions of desoxycholic acid. The formation or non-formation of a choleic acid under these conditions is dependent merely upon the relative solubilities of the components and of the complex and upon the degree of association of the coordinate compound. A dilute solution of methylcholanthrene-choleic acid gives the spectrum of the pure hydrocarbon, indicating that the molecular compound is completely dissociated.

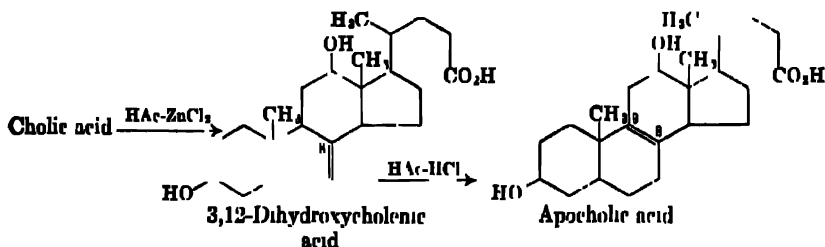
The choleic acids dissolve in dilute alkali, on the other hand, without dissociation into the components. Water-insoluble substances, including fats and higher aromatic hydrocarbons, can be brought into aqueous solution in the form of the sodium salts of the choleic acids. On discovering this remarkable phenomenon, Wieland and Sorge suggested that the dissolving power of the bile may be due to the "choleic acid principle." While the hypothesis is an attractive one, there are reasons for questioning the importance of molecular compound formation in the process of fat resorption. Desoxycholic acid is not the most abundant constituent of animal biles, and the other naturally occurring bile acids do not share the property of forming choleic acids. Furthermore desoxycholic acid is not present in bile in the free condition but in conjugation with glycine and taurine, and it is questionable if the natural conjugated bile acids form coordinate compounds of stability at all comparable with that of the desoxycholic acid compounds.⁷⁶ The sodium salts of the conjugated bile acids are responsible for the pronounced power of the bile to dissolve water-insoluble fats, but the substances apparently act by surface forces and actual compound formation probably is of little significance.

The unique character of the choleic acids suggests interesting applications which have been only partially explored. Sobotka found that choleic

⁷⁵ Sekitoo, *Z. physiol. Chem.*, 199, 325 (1931).

acid formation favors the keto \rightarrow enol change,⁷⁹ and further experiments of this investigator suggest the possibility of resolving *dl*-mixtures of chemically inert compounds by crystallization of the choleic acids.⁸⁰ Substances acquire considerably modified properties when present in coordinate combination, for example benzaldehyde-choleic acid is not subject to autoxidation. The resorption in the alkaline intestinal tract of alkaloid drugs which are water-insoluble, may be dependent upon the choleic acid principle (Wieland). The molecular compound of camphor with desoxycholic acid has been employed as a pharmaceutical preparation ("Codechol").

Although the other acids obtained from bile do not form stable choleic acids, the special property of desoxycholic acid is shared by the transformation product apocholeic acid. Boedecker⁸¹ obtained this substance,



together with the isomeric 3,12-dihydroxycholelenic acid, by the action of mild dehydrating agents on cholic acid, and Yamasaki⁸² showed that 3,12-dihydroxycholelenic acid rearranges to apocholeic acid in the presence of strong acids even at room temperature. The rearrangement involves a migration of the double bond from an active to an inactive position, for the more labile isomer can be hydrogenated, yielding desoxycholic acid, while apocholeic acid is not susceptible to catalytic hydrogenation. From these and other observations, Wieland and Dane⁸³ were able to establish fully the structure of 3,12-dihydroxycholelenic acid and to show that the double bond of apocholeic acid probably is located between the two bridge heads C_8 and C_9 . The isomers differ from desoxycholic acid only in having one ethylenic linkage in the otherwise saturated ring system, and it is very interesting that apocholeic acid is capable of forming stable choleic acids while this property is not shared by 3,12-dihydroxycholelenic acid. Possibly it is the inactive character of the double bond of apocholeic acid rather than its specific position which allows the substance to function like the saturated, naturally occurring acid.

⁷⁹ Bobotka and Kahn, *Biochem. J.*, **26**, 898 (1932), *Ber.*, **65**, 227 (1932).

⁸⁰ Bobotka and Goldberg, *ibid.*, **26**, 905 (1932).

⁸¹ Boedecker, *Ber.*, **53**, 1852 (1920), Boedecker and Volk, *ibid.*, **54**, 2489 (1921).

⁸² Yamasaki, *E. physiol. Chem.*, **233**, 10 (1935).

⁸³ Wieland and Dane, *ibid.*, **212**, 263 (1932).

INVESTIGATIONS OF STRUCTURE⁵⁴

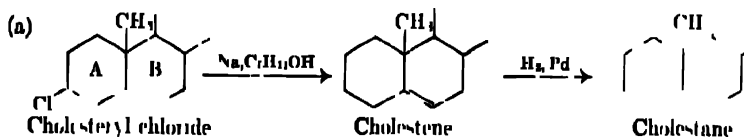
The Nature of the Problem. The investigations directed to the elucidation of the structures of the sterols and bile acids were beset by a conspiracy of adverse factors. The structural formulas eventually assigned show that the problem was inherently difficult, for the substances are of a very complicated type for which there was scarcely any previous parallel. Hydrogenated polynuclear compounds were not available by synthesis for comparison studies. When it is considered that cholic acid contains no less than eleven asymmetric carbon atoms it is clear that the possibility for stereoisomerism is enormous. The natural products often crystallize quite well when they are perfectly pure, but the process of crystallization can be thrown completely out of gear by the presence of small amounts of foreign bodies. Since the large molecules offer particular opportunity for the occurrence of side reactions, transformation products usually must be separated from mixtures by repeated, slow crystallizations. The yields are often very poor, and it is a matter of particular interest that it was because of the difficulty experienced in obtaining a certain bile acid degradation product in quantity sufficient for characterization that Pregl undertook the development of his classical methods of microanalysis. These methods proved to be of inestimable service in the subsequent investigations in this and other fields. Another source of difficulty and delay in working with the bile acids is associated with the tendency of these substances to form molecular compounds and to retain solvents. It may be said finally that the long and arduous study of a succession of compounds of much the same crystalline character was at no point either assisted or enlivened by the appearance of colored substances.

Early Observations. Three phases may be distinguished in the investigations of the sterols and bile acids, the first being that in which attention was directed chiefly to the isolation, analysis, and characterization of the compounds. Accurate analyses of cholesterol were carried out by Chevreul in 1823, and in 1859 his results were recalculated to the modern basis by Berthelot, who suggested the formula $C_{26}H_{44}O$, for which the theoretical composition is: C, 83.80; H, 11.91. This is remarkably close to the correct formula $C_{27}H_{46}O$ (C, 83.86; H, 11.99) established by Reitner in 1888 from the analysis of halogen derivatives, which offer a better means of discrimination. Early work on the nature of the constituents of bile and of gall stones was reported from the laboratories of Gmelin, Thénard, Berzelius, and Liebig, but the first isolation of pure substances

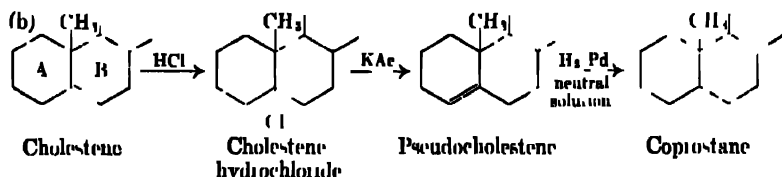
⁵⁴ For review papers see Windaus, *Z. physikal. Chem.*, 213, 147 (1933); Wieland, *Ber.*, 69A, 27 (1934); Dane, *Z. anorg. Chem.*, 47, 351 (1934); Windaus, *Ann. Rev. Biochem.*, 1, 109 (1932); Rosenheim and King, *ibid.*, 3, 57 (1934); Sobotka, *Chemical Reviews*, 15, 311 (1934).

was reported in 1848 by Strecker, who obtained the conjugated acids *glycocholic acid* and *taurocholic acid*. In 1886 Mylius isolated *desoxycholic acid* from hydrolyzed bile and Hammarsten soon obtained *taurodesoxycholic acid*. "Cholic acid," the supposed isomer of *desoxycholic acid*, was isolated by Latschinoff in 1885.

Of the earlier findings regarding the nature of the functional groups, only a few of the more significant observations will be outlined. In the case of cholesterol the presence of the hydroxyl group was established by Berthelot (1859) by the preparation of acyl derivatives, and Diels and Abderhalden⁵⁵ in 1903 showed this to be a secondary alcoholic group by the dehydrogenation of cholesterol to cholestenone. Wislicenus and Moldenhauer⁵⁶ (1868) established the presence of the double bond by the formation of a dibromide, and the parent hydrocarbon, *cholestane*, was obtained by Mauthner and by Diels (1907-1909).⁵⁷ One route to the saturated hydrocarbon is by way of the chloride of cholesterol, as shown in the formulas (a). The stereoisomer *coprostane* (or pseudo-



cholestane) was prepared from an isomerization product of cholestene (b)



While the hydrogenation of pseudocholestene in neutral solution yields principally coprostane, cholestane is the exclusive product when an acidic medium is employed. Coprostane can be obtained also by the reduction of coprosteryl chloride with sodium and amyl alcohol.⁵⁸

Since cholestane and coprostane are saturated compounds, the empirical formula $\text{C}_{27}\text{H}_{48}$, in comparison with that for an open chain paraffin hydrocarbon ($\text{C}_{27}\text{H}_{56}$), indicated the presence of four rings in the cholesterol molecule. The same inference was drawn in the case of the bile acids. The functional groups of these substances were characterized by

⁵⁵ Diels and Abderhalden, *Ber.*, **36**, 3177 (1903); **37**, 3002 (1904).

⁵⁶ Wislicenus and Moldenhauer, *Ann.*, **146**, 175 (1868).

⁵⁷ Mauthner, *Monatsh.*, **28**, 1118 (1907); **30**, 635 (1909). Diels and Linn, *Ber.*, **41**, 514 (1908).

⁵⁸ Windaus and Uhlig, *Ber.*, **48**, 867 (1915).

orthodox methods and the parent substance cholic acid was obtained in the manner already described.

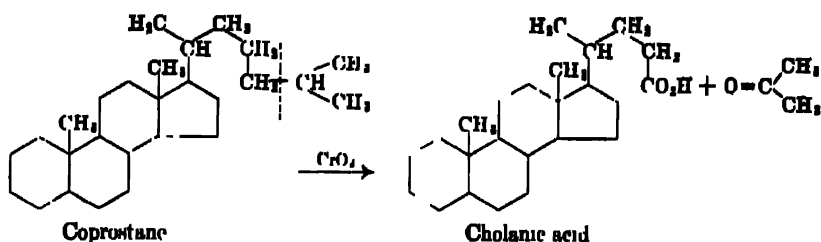
The Method of Attack. In the second phase of the study an insight into the structures of the complicated molecules was sought through an examination of various products of oxidation, and the most important contributions to this part of the work are associated with the names of Borsche, Diels, Mauthner, Schenck, Wieland, and Windaus. Of outstanding brilliance was the work of Windaus on cholesterol dating from 1903⁸⁹ and that initiated by Wieland⁹⁰ in 1912 in the field of the bile acids. The long series of investigations at Gottingen, and at the Freiburg and Munich laboratories, followed for a time parallel but quite independent paths and the method of attack in each case was by degradation. To attempt to approach the problem by the synthesis of substances which might be related to the complicated natural products was out of the question, not so much because adequate synthetical methods would have to be developed, but because there was no characteristic property to serve as a guide to such work. H. Fischer was able to investigate the blood pigments from the synthetic side because the highly distinctive absorption spectra of the porphyrins made it possible to determine when the synthetic attempts were leading in the right direction. Such a method was excluded in the case of the colorless, and for the most part saturated, bile acids and sterols. Conversion to an aromatic substance by dehydrogenation, a method which would be expected to give valuable information as to the carbon skeleton and which was of such service in the case of the resin acids, probably was tried many times with negative results, for the dehydrogenation presents unusual experimental difficulties. When eventually success was achieved in 1927 the results formed an important link in the chain of evidence, but this work belongs to the most recent phase of the investigations and will be discussed later.

Attempts to determine the character of the ring systems were made at an early date in the studies of oxidative degradation, but this proved to be the most difficult part of the problem. The elaboration of the character of the side chains was by no means simple, but this part of the work was the first to be carried to a sure conclusion and it affords the more convenient starting point for a description of some of the advances made by the German investigators.

The Characterization of the Side Chain. The ready oxidation of both the sterols and the bile acids had invited early attempts to characterize the host of oxidation products which can be obtained by operating under various conditions. It had been observed as early as 1872 that

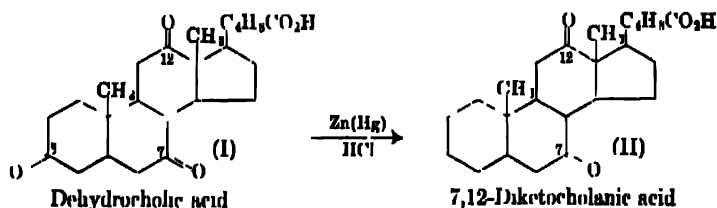
⁸⁹ Windaus, *Ber.* 36, 3752 (1903)

⁹⁰ Wieland and Weil, *Z. physiol. Chem.* 80, 267 (1912)



The Hydroxycholanolic Acids. In the difficult task of characterising the four carbocyclic rings, the secondary alcoholic groups served as indispensable handles with which to manipulate the complicated structures. A ring containing such a group could be opened by oxidation at the vulnerable point and its character investigated. In the case of the bile acids several natural substances were available for experimentation and as the work unfolded methods were discovered for converting cholic acid into naturally occurring mono- and di-hydroxycholanolic acids and for proving that the location of the hydroxyl groups in these acids is largely limited to three positions. It also became possible to obtain for use in oxidative degradations certain other hydroxy acids or the corresponding keto acids, and a few examples will illustrate the methods of transformation.

The hydroxycholanolic acids can be converted smoothly into the corresponding keto ("dehydro") acids by oxidation with chromic anhydride in glacial acetic acid solution at room temperature, and such differences as exist in the rate of attack, depending upon the positions of the hydroxyl groups, are not marked. The ease of reduction of the keto acids by the Clemmensen method, however, varies greatly with the location of the carbonyl groups. Borsche²⁵ found that the 3-keto group of dehydrocholic acid (I) can be reduced to a methylene group under conditions which leave ketonic groups at C₇ and C₁₂ unchanged:

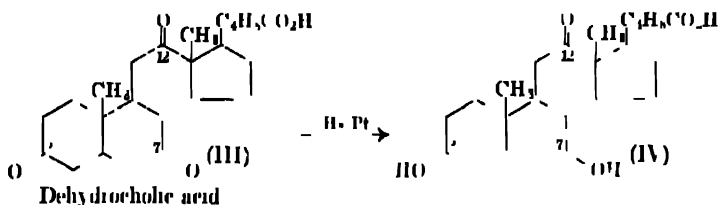


The greater reactivity of the group at C₃ may be attributed to the fact that this occupies a β -position in the ring and that, unlike the C₇ and C₁₂ groups, it is not subject to the steric interference of adjacent rings or substituents. In a similar manner, but using unamalgamated zinc,

²⁵ Borsche, *Ber.*, 52, 1363 (1919).

Wieland and Schlichting⁸⁶ reduced the 3,12-diketo acid, dehydrodesoxycholic acid, to 12-ketocholanic acid, the oxygen atom at C₃ again being eliminated. The same selectivity is apparent in the addition of hydrogen to the carbonyl group, for Borsche⁸⁷ was able to convert the 3,7,12-triketo acid by treatment with sodium amalgam into 3-hydroxy-7,12-diketocholanic acid, which yielded lithocholic acid on reduction by the Wolff-Kishner method, that is, by the action of sodium ethylate on the disemicarbazone. The same result was later accomplished by a series of transformations starting with the conversion of dehydrocholic acid into the 3,3-dichloro-7,12-diketo acid by the action of phosphorus pentachloride.⁸⁸

A distinction between the reactivity at the 7- and 12-positions is afforded by the observation of Kawai⁸⁹ that there is a marked break in the hydrogenation of the 3,7,12-triketo acid (III) after the absorption of two moles of hydrogen and that the 3,7-dihydroxy-12-keto acid (IV) is the chief reaction product. Since there is both a ring and a methyl group



adjacent to the carbonyl group at C₁₂, the decreased reactivity as compared with the group at C₇ is easily understandable from steric considerations. Kawai made a useful application of this differentiation. The semicarbazone of the keto acid IV was converted by treatment with sodium ethylate into 3,7-dihydroxycholic acid, which was found to be identical with chenodesoxycholic acid.

That the order of reactivity C₃ > C₇ > C₁₂ holds in reactions other than reduction is clearly shown in an interesting series of transformations accomplished by Wieland and Kapitel.¹ Cholic acid was converted by partial acetylation into the 3,7-diacetate, V, which gave the ketone VI on oxidation. By partial hydrolysis, the acetyl group at the more reactive C₃-position could be split off, giving 3-hydroxy-7-acetoxy-12-ketocholanic acid, VII. By oxidizing this to the 3,12-diketone and reducing the carbonyl groups by the Wolff-Kishner method, VII was transformed into 7-hydroxycholic acid, VIII. 12-Ketocholanic acid, X, was obtained

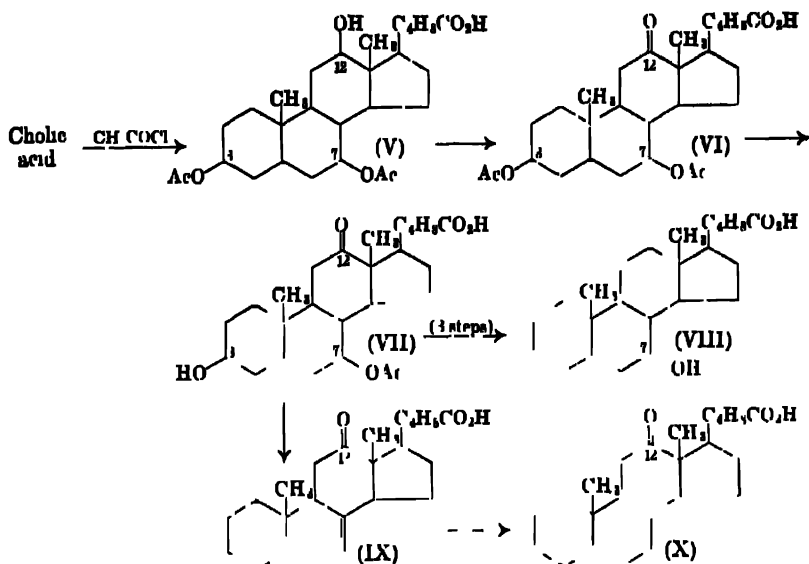
⁸⁶ Wieland and Schlichting, *Z. physiol. Chem.*, **150**, 267 (1925)

⁸⁷ Borsche and Hallwax, *Ber.*, **55**, 1314 (1922)

⁸⁸ Borsche and Morrison, *Z. physiol. Chem.*, **198**, 165 (1931)

⁸⁹ Kawai, *ibid.*, **214**, 71 (1933)

¹ Wieland and Kapitel, *ibid.*, **212**, 264 (1932)



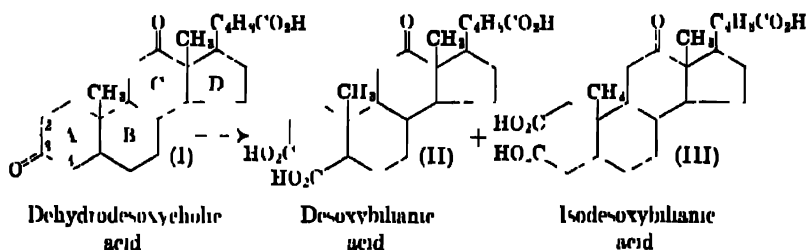
from VII through the 12-ketocholadienic acid, IX, which was prepared by pyrolysis and which yielded the saturated keto acid on hydrogenation. It is evident that by a suitable selection of methods it is possible to transform the cheap cholic acid into any of the mono- or di-hydroxy acids² substituted in positions C, C₇, and C₁₂. The methods all depend upon the gradation in the reactivity at these positions, for this is apparent in reactions of acetylation, hydrolysis, oxidation, reduction, and hydrogenation. The only exception reported is that 3,7-diketocholanic acid (dehydrocheno acid) yields 3-hydroxycholanic acid (litho acid) when the monosemicarbazone is heated with sodium ethylate,³ but the reaction is somewhat anomalous in other respects since the free carbonyl becomes reduced to an alcoholic group.

The Characterization of Ring A. Having available for experimentation so many different but interrelated hydroxyl derivatives of cholanic acid, the investigators in the field were able to open the complicated ring structure by oxidation at various different points and so effect degradations which furnished much information as to both the ring system and the location of the alcoholic groups. The work in this direction has been so very profuse that it is only possible in a limited amount of space to give an indication of the nature of the experimental methods and to summarize the more important conclusions.

² Tathocholic acid is prepared more conveniently from desoxycholic acid, through the δ -acetyl derivative (prepared by partial acetylation) and 3-acetoxy-12-ketocholanic acid [Wieland, Dane and Scholz, *Z. physiol. Chem.*, 211, 266 (1932)].

³ Wieland and R. Jacobi, *Z. physiol. Chem.*, 148, 242 (1925).

The hydroxycholanolic acids or reduced sterols can be converted by mild oxidation into the corresponding keto compounds and on further oxidation, usually with either concentrated nitric acid or potassium permanganate, the ring containing the carbonyl group is opened with the production of two new carboxyl groups. In the bile acid series the tri-carboxylic acid obtained by the opening of a single ring is called a bilianic acid and a prefix (desoxy-, litho-, cheno-, etc.) is used to indicate the series in question. In the oxidation of the diketone acid (I) from desoxycholic acid the reactive group at C_3 forms the first point of attack and the ring is cleaved both between C_3 and C_4 (chief product, normal series) and between C_2 and C_3 (iso-series), giving rise to two acids (II and III).⁴



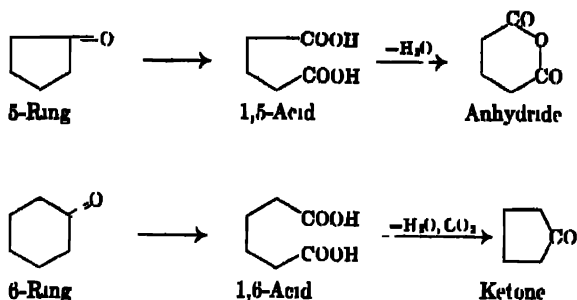
The formation of tribasic acids as the main products reveals the presence of a methylene group adjacent to the carbonyl group in the ring attacked ($-\text{CH}_2-\text{CO}-$), and the isolation of an isomer, coupled with the independent demonstration that it does not arise from the opening of ring C and that it is not a stereoisomeride, indicates that the reactive group is flanked on each side by methylene groups ($-\text{CH}_2-\text{CO}-\text{CH}_2-$). This identifies the ring in question (A) as being at the end of the molecule, for a ring situated in the manner of either B or C could open in only one way. The evidence also serves to locate the carbonyl group at a β -position in ring A, namely at C_1 or C_3 .

The size of ring A was inferred from a further transformation of the desoxybilianic acids, namely the cyclization by distillation. From regularities observed among simple dibasic acids, Blanc⁵ had formulated a rule which Windaus⁶ first applied to this type of oxidation product in 1919 and which has since occupied a position of great prominence in the study of structure. The Blanc rule states that if the two carboxyls occupy the 1,3-, 1,4- or 1,5-positions in the chain the acid on treatment with acetic anhydride (or on distillation) is converted into an anhydride, while 1,6- and higher acids yield cyclic ketones. The most pertinent applications to the present problem are in the cases of the acids resulting

⁴ Wieland and Kulenkampff, *Z. physiol. Chem.*, 108, 215 (1920).

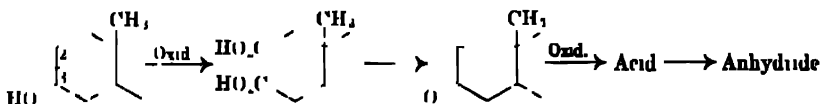
⁵ Blanc, *Compt. rend.*, 144, 1356 (1907).

⁶ Windaus and Dalmor, *Ber.*, 52, 162 (1919).



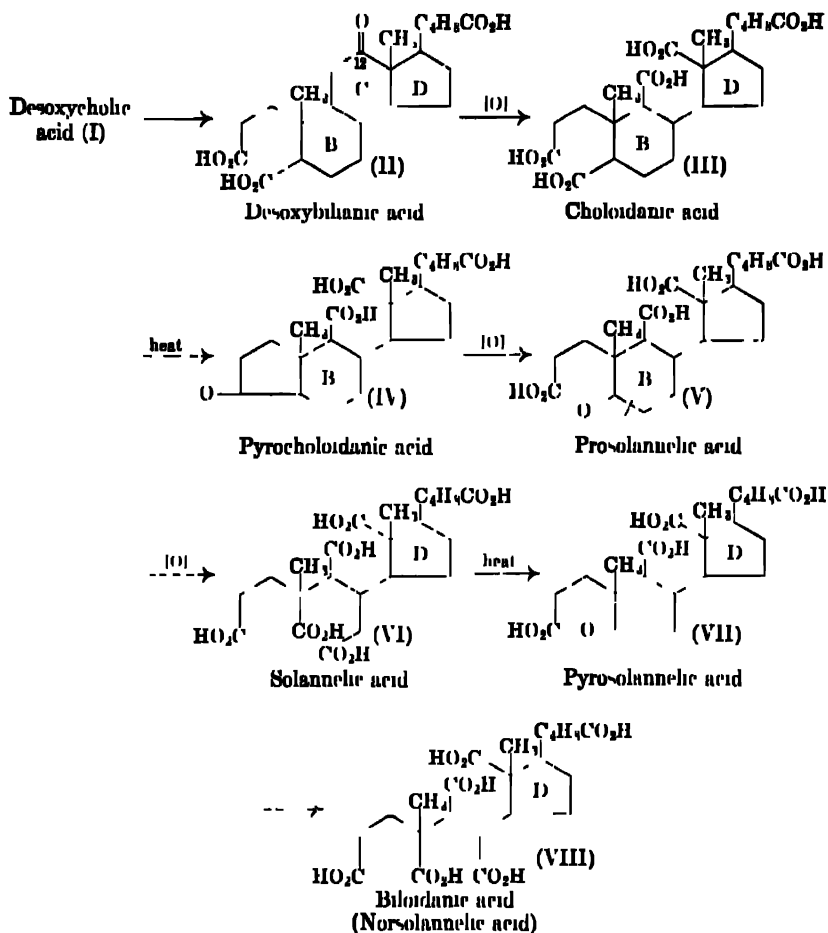
from the opening of five- and six-membered rings. Both desoxybilianic acid and its isomer on distillation lose carbon dioxide and water and form ketones, indicating that ring A of the original bile acid is a six-membered ring.

In the sterol series the same conclusion was reached⁶ regarding the ring (A) containing the lone alcoholic group, and the evidence was carried a step further. The acid resulting from the oxidation of dihydrocholesterol yields a ketone on pyrolysis, while the acid from the pyroketone gives an anhydride. According to the Blanc rule both observations clearly indicate an original six-ring.



That ring A of dihydrocholesterol is opened largely between positions 2 and 3 was established by later evidence (Windaus,⁴ 1933). The oxidation follows a course different from that observed in the case of the bile acids, where cleavage occurs chiefly at $\text{C}_3\text{-C}_4$. The difference appears to be connected with the fact that rings A and B of the bile acids have the *cis* configuration while cholesterol belongs to the *trans* (*allo*) series. Coprosterol (A/B *cis*) is attacked chiefly at the 3,4-position.

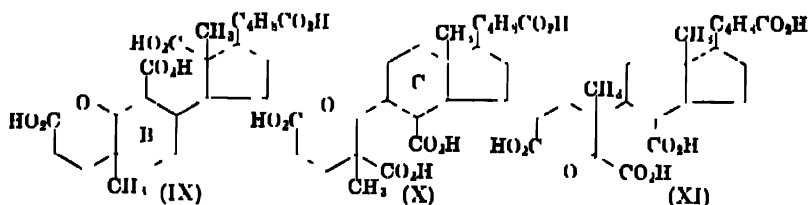
The Evidence Regarding Ring B. The rule of Blanc was not at first employed in a similarly direct manner to the investigation of the size of ring B of the bile acids because various other observations furnishing information on this point became available before methods suitable for the selective opening of ring B had been developed. One piece of evidence emerged from a study of the further degradation of desoxycholic acid, a complete account of which is given in the accompanying formulas. When desoxybilianic acid (II) is oxidized ring C is cleaved adjacent to the keto group at C_{12} and on pyrolysis of the reaction product, choloidanic acid (III), cyclization with loss of carbon dioxide takes place in the part



of the molecule corresponding to the original ring A, giving IV (in the form of the anhydride). The new ketonic ring is cleaved on oxidation of the pyroacid and, since the bond severed makes connection to a tertiary carbon atom rather than to a methylene group, the acid V has only one new carboxyl group and is a keto acid. Prosolannelic acid (V) forms the starting point for the opening of ring B, which originally carried no hydroxyl groups, and the Blanc reaction can be applied to the oxidation product VI which, since only one of the original rings (D) is still intact, is called solannelic acid (*L. solus annulus*). Since carbon dioxide is lost in the formation of the ketonic pyroacid VII, it was concluded that B is a six-membered ring.

A careful analysis of the series of degradations furnishes still further information. The evidence given above located the hydroxyl group of

ring A at either C₂ or C₁, and in the formulas it has been placed at C₂. The alternate location would require a modification in the structures in such a way that prosolannelic acid (V) would acquire the formula IX. This structure is inadmissible, however, because prosolannelic acid does



not have the properties of a β -keto acid as demanded by the formula.⁷ This observation rules out other structures for the original desoxychohic acid. If one hydroxyl were at C₂ or at C₃, and if the second were located in ring B, prosolannelic acid would have the structure of X, which is a β -keto acid, or the equally inadmissible structure, XI, of an α -keto acid. The location of the hydroxyls at C₁ and C₂ (or at C₂ and C₁₁) is excluded on similar grounds, and the degradations indeed furnish good evidence that B is a six-membered ring, that one hydroxyl is at C₁₁, and that the second one is attached to ring C. Corroborative evidence is not lacking to establish the order in which the rings open. For example desoxybilianic acid and isodesoxybilianic acid can be reduced by the Wolff-Kishner method to the carbonyl-free acids, which are identical with lithobilianic acid and isolithobilianic acid, respectively.⁸ Since the conversion of lithochohic acid into these isomeric acids can only be the result of the opening of the terminal ring, this proves that it is ring A which is cleaved in the formation of the desoxybilianic acids.

Another line of evidence regarding the nature of ring B was developed by Borsche.⁹ A number of investigators had studied the oxidation of dehydrochohic acid, Pregl¹⁰ for example obtaining bilianic acid (XII) and isobilianic acid in yields of 44 per cent and 2 per cent, respectively. Lassar-Cohn¹¹ had obtained from bilianic acid with alkaline permanganate another oxidation product which he called cilianic acid, and this substance had been investigated extensively by Wieland and Schlichting,¹² who cleared up many points but failed to arrive at the true interpretation of the formation and degradation of cilianic acid. According to Borsche

⁷ Wieland and Schulenberg, *Z. physiol. Chem.*, **114**, 107 (1921).

⁸ Borsche and Hallwax, *Ber.*, **55**, 3314, 3324 (1922); Borsche and Brhl, *Nachr. Ges. Wiss. Göttingen*, **188** (1920).

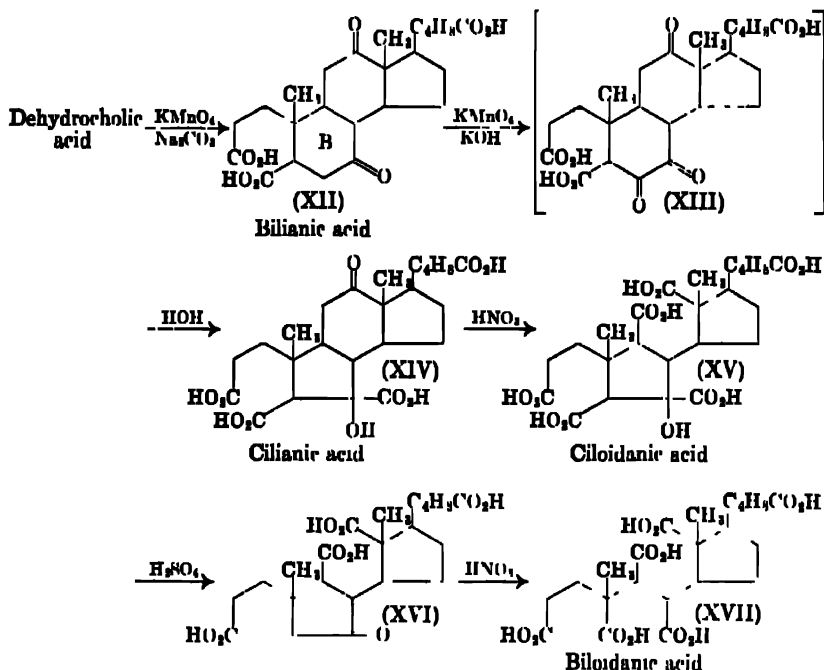
⁹ Borsche and Frank, *Ber.*, **60**, 723 (1927).

¹⁰ Pregl, *Monatsh.*, **24**, 19 (1903).

¹¹ Lassar-Cohn, *Ber.*, **32**, 683 (1900).

¹² Wieland and Schlichting, *Z. physiol. Chem.*, **120**, 227 (1922); **123**, 214 (1922).

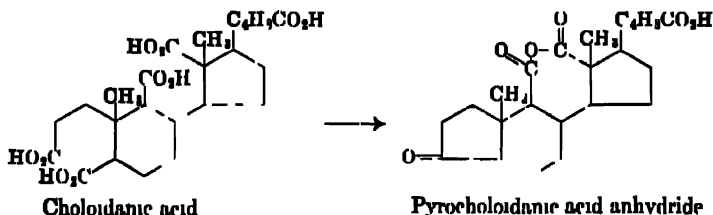
the first step in the oxidation consists in the formation of the unstable triketo acid XIII, which undergoes the benzilic acid rearrangement in the alkaline solution and yields cilianic acid (XIV):



The presence of the various functional groups was established by orthodox methods and their arrangement was inferred largely from the behavior of the oxidation product, ciloidanic acid (XV), when warmed with concentrated sulfuric acid. Carbon monoxide and water are eliminated from the α -hydroxy acid group ($>\text{C} < \begin{smallmatrix} \text{OH} \\ \text{COOH} \end{smallmatrix} \longrightarrow >\text{C}=\text{O} + \text{CO} + \text{H}_2\text{O}$)

and the resulting β -keto acid then easily becomes decarboxylated, giving XVI. The last oxidation product (XVII) was found identical with biloidanic acid, obtained as described above from desoxycholic acid. If B is a six-ring, as pictured in the formulas, the transformation of this into a five-membered ring by way of a benzilic acid rearrangement is readily understandable, but it would not be admissible to assume that the new ring formed in this facile manner contains only four carbon atoms and hence that it comes from an original five-membered ring. The degradations lend further support to the structure attributed to the second ring of cilianic acid, and the observations taken as a whole provide reliable evidence that ring B in the bile acids is a cyclohexane ring.

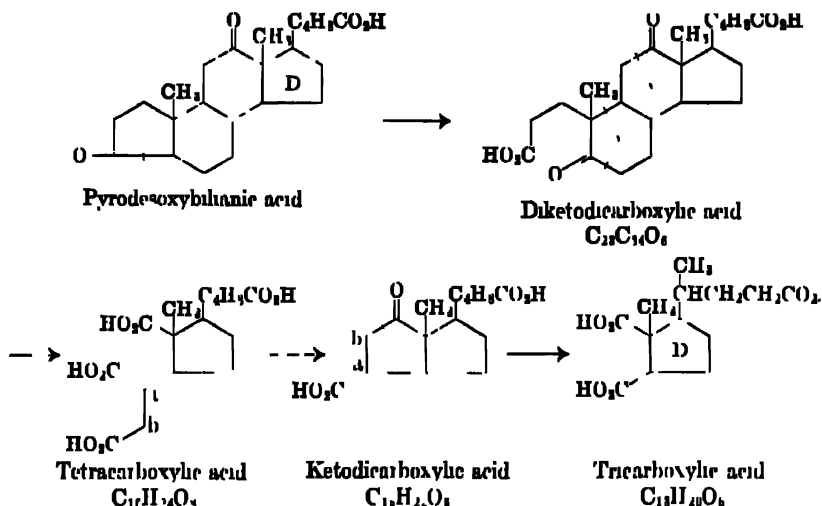
The Failure of the Blanc Rule. The evidence available from the early work concerning ring C was limited to a single observation by Wieland¹⁸ in 1920 regarding choloidanic acid, and this unfortunately led to a conclusion which was later found to be erroneous. Choloidanic acid, the oxidation product of desoxycholic acid in which both rings A and C have been opened, yields on pyrolysis an acid containing two new rings, a ketonic ring coming from the residue of A and an anhydride ring formed from the carboxyl groups originating from the cleavage of C. In accordance with the Blanc rule, Wieland took this to mean that rings A and C contain six and five carbon atoms, respectively. Although the assumption was plausible enough at the time, it is now known that C is a six-membered ring. As will be shown later the Blanc rule does not hold for



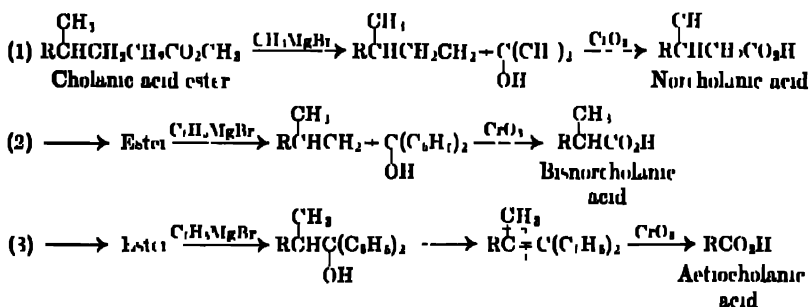
ring C, possibly because of a peculiar configuration of the complicated molecule, possibly because both of the carboxyl groups are attached to rings. Because of the apparent success in the application of the method of diagnosis in other cases, the validity of the Blanc rule was not at first questioned and the erroneous conclusion remained uncontested for a number of years. The manner in which the mistake was discovered will be described after completing a survey of the early work.

The Character of Ring D. The investigation of the fourth ring, D, presented special difficulties because no bile acid or sterol having an oxidizable group in this part of the molecule has been discovered. One plan of attack pursued for a time by Wieland was to destroy all of the other rings by oxidation in the search for a degradation product suitable for the characterization of the remaining ring. The closest approach to the realization of this project was in the degradation of pyrodesoxybilianic acid, page 146.¹⁴ The acid $C_{13}H_{20}O_6$ obtained as the end product might have served as a satisfactory starting material for the investigation of ring D if the procedure for obtaining it had been less laborious and if the yield had not been in the order of 5 g. from 1 kg. of desoxycholic acid. The difficulties were so great, however, that Wieland eventually turned to another line of approach and sought a means of opening the ring in question by the

¹² Wieland, *Z. physiol Chem*, 108, 806 (1920)²⁴ Wisland and Schlichting, *ibid.*, 134, 276 (1984).



stepwise degradation of the bile acid side chain. A number of the known methods of degradation were found to be quite useless when applied to the complex bile acid molecule, but the objective was finally achieved by Wieland, Schlichting and Jacobi¹⁷ in 1926 by the successive oxidation of the carbinols obtained by the Grignard reaction from the esters of cholanic acid and its lower homologues, as follows:

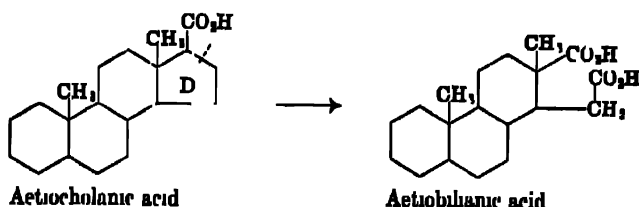


This work incidentally confirmed the structure already assigned to the side chain, for the loss of a single carbon atom in steps (1) and (2) proves the presence of two methylene groups next to the acid group, and the branching methyl group is revealed by the elimination of two carbon atoms in (3).

It was evident that the base of the chain had been reached in the formation of aetiocolanic acid (Gr. *aetio*-, fundamental) for on further oxidation this was converted into a dibasic acid, aetio-bilanic acid, which

¹⁷ Wieland, Schlichting and R. Jacobi, *Z. physiol. Chem.*, **161**, 80 (1926).

could only arise from the opening of the ring carrying the acid group. The opening of ring D was in this way realized and although the overall

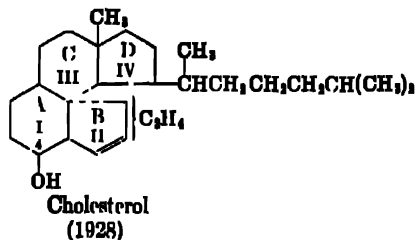
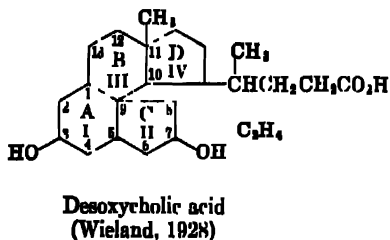


yield of aetiobilanic acid was considerably less than 1 per cent it was possible to characterize the substance by means of the Blanc reaction. The acid lost no carbon dioxide on being distilled but formed an anhydride, from which it was correctly concluded that the ring in question contains five carbon atoms. The conclusion was at the time quite unexpected.

The "Old" Formulas. Without mentioning a host of other observations in the field of the bile acids and the sterols alike it may be said that by 1928 the work had reached a point where the structural problem appeared to be settled in all of the most essential points, and the award of the Nobel Prize of that year to Wieland and to Windaus was a fitting tribute to the brilliant success in the arduous investigations in a most difficult field. The formulas suggested in 1928 hardly reflect the real progress which had been made, for they were unduly distorted by the errors which had crept into the train of evidence. One error arose from the failure of the Blanc rule when the carboxyl groups concerned are located between two ring systems. Another was in inferring from certain of the degradations¹³ that the supposedly five-membered ring C which carries the second hydroxyl group of desoxycholic acid is attached to ring A and shares two carbon atoms in common with this ring. It is unfortunate that this assumption remained for many years unchallenged, for it really could not be justified on the basis of the experimental evidence available. That ring B is directly joined to ring A, as in the formulas now accepted, had been established quite definitely. The oxidation of prosolannelic acid to solannelic acid for example proves that the oxygen-free ring (B) of desoxycholic acid is attached to the ring (A) which is opened first in the oxidation of the bile acid.

The combination of the correct and the mistaken evidence led to the construction of a curious formula¹⁴ in which the three rings in question were pictured as being directly united. In the older literature the four rings were indicated by Roman numerals in the manner shown; the

¹⁴ Wieland, *Z. angew. Chem.*, 42, 421 (1929).



identity of the rings in terms of the modern structure is indicated by the letters. The numbering of the positions is such that, while C_1 is identical in the old and the new formulas, the C_7 position is now called C_{12} , and vice versa. Desoxycholic acid, known in the early literature as "3,7"-dihydroxycholanic acid is now recognized as the 3,12-acid. The tentative formula for cholesterol was based in large part upon that assigned to the bile acids, for the latter offered more points of attack by oxidative degradation. The old ring II which contains the double bond was supposed to be identical with the old ring II of the bile acids because both rings showed the same behavior in the pyro-reaction.¹⁵ Both rings were regarded as five-membered rings because anhydrides were produced, but in each case the results are contrary to the predictions of the Blanc rule and the rings in question are now known to be different (B and C). In both the old and the new formulas for cholesterol the double bond is placed in the β,γ -position with respect to the hydroxyl group, but the latter has been shifted from C_4 to C_3 in the light of recent evidence.

The placing of a methyl group at C_{11} in the old formula was not without some experimental foundation, but this left two "homeless" carbon atoms for which no satisfactory account could be given. Wieland's tentative hypothesis that an ethyl group is located at C_{10} (old formula) was later found to be inadmissible¹⁷ and the insertion¹⁸ of the grouping $-\text{CH}(\text{CH}_3)-$ between C_1 and C_9 or between C_{11} and C_{12} could not be supported for long and it proved to be impossible to place the "homeless" carbon atoms anywhere in the ring system.

The Problem of Revision. With the realization of these difficulties in adding the finishing touches to a picture which in 1928 had appeared to be practically complete, the way was thrown open to a consideration of a modified formulation for the bile acid ring skeleton, and at this point the investigations may be said to have entered a third phase, that of revision. The first indication of the direction which this revision would have to take came from an unexpected source. Bernal¹⁹ at the Mineralogical

¹⁵ Wieland and Posternak, *Z physiol Chem*, **197**, 17 (1931)

¹⁶ Wieland and Voelke, *ibid*, **191**, 69 (1930), Wieland and Dane, *ibid*, **206**, 243 (1932).

¹⁷ Bernal, *Nature*, **129**, 277 (1932)

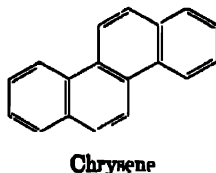
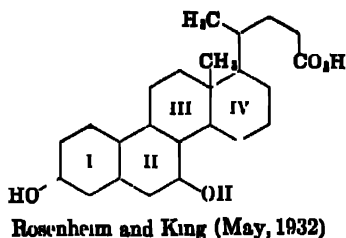
Museums, Cambridge, made an X-ray crystallographic examination of ergosterol and of various of its irradiation products in 1932 with the primary object of defining the position in the series of vitamin D, and he made the incidental observation that ergosterol molecules form double layers similar to those of long-chain alcohols and that the molecular dimensions do not fit at all well with the values calculated from the Wieland-Windaus sterol formula. This represents three rings as meeting at a single point (C_3), and the molecule would necessarily be rather thick, but Bernal's evidence pointed to a long, thin molecule. Bernal's conclusion that the results were difficult to reconcile with the accepted structure had the support of a previous but less specific observation of Adam and Rosenheim,²⁰ who had investigated the surface films formed by certain of the sterols.

In speculating on the matter of the molecular dimensions and seeking some new formulation which would accord better with the X-ray measurements, Rosenheim and King,²¹ at the National Institute for Medical Research, London, took a clue from an important but little-considered observation reported by Diels in 1927.²² Diels had discovered that the sterols and bile acids can be dehydrogenated by the action of palladium charcoal at a high temperature (ca. 500°) or, better, by prolonged heating of the substances with selenium at a more moderate temperature (360°), and he had isolated from some of the resulting mixtures small amounts of three aromatic hydrocarbons. Two of these had not been identified in 1932, but the third substance had been recognized as chrysene. The formation of this hydrocarbon from cholesterol represents the loss of no less than nine carbon atoms, and it seemed by no means unlikely that a deep-seated rearrangement in the ring system also occurs under the rather brutal conditions of the reaction. The Wieland-Windaus conception of the cholane skeleton appeared at the time to be so firmly established that Diels, Wieland, and most other investigators were inclined to the view that the chrysene arises as the result of a drastic rearrangement, rather than that the reaction affords any reliable indication of the nature of the original ring system. When the old formulation became subject to serious doubt as the result of the X-ray studies, it occurred to Rosenheim and King that chrysene may be a normal degradation product, and on this basis they constructed an entirely novel cholane formula, which may be illustrated for the case of desoxycholeic acid (see page 150). Ring III was moved to the other side of ring II and the two "homeless" carbon atoms were accommodated by enlarging rings II and IV to six-

²⁰ Adam and Rosenheim, *Proc. Roy. Soc. (London)*, **A126**, 25 (1926).

²¹ Rosenheim and King, *Chemistry and Industry*, **51**, 481 (1932).

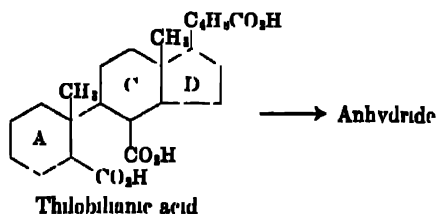
²² Diels and Gadke, *Ber.*, **60**, 110 (1927); Diels, Gadke and Kording, *Ann.*, **459**, 1 (1927).



membered rings. The agreement with the X-ray data was now quite satisfactory, as shown by comparisons given by Bernal:²³

Ergosterol dimensions	Found	$7.2 \times 5 \times 17.20 \text{ \AA}$
	Wieland-Windaus formula	$8.5 \times 7 \times 18 \text{ \AA}$
	Rosenheim and King formula	$7.5 \times 4.5 \times 20 \text{ \AA}$

In an investigation completed two months after the appearance of Rosenheim and King's paper, an observation was made by Wieland and Dane²⁴ which revealed for the first time the uncertainty in the determination of the size of a ring by the Blanc method. It already was known²⁵ that lithobilianic acid, in which ring A has been opened, yields a ketone on pyrolysis and that an isomeric acid, formed by oxidizing 12-hydroxycholanic acid and thus cleaving ring C, gives an anhydride, in conformity with the then accepted view that rings A and C contain six and five atoms, respectively. On investigating the third isomer, called thilobilianic acid (transposition of "litho") to indicate the relationship



to the litho compounds, Wieland and Dane were surprised to find that the pyrolysis product is an anhydride. According to the Blanc rule this would imply that ring B is a five-membered ring, but convincing evidence was already on record characterizing this as a six-ring, in particular that furnished by Borsche's work on cilianic acid. Indeed the evidence regarding ring B from the behavior of thilobilianic acid is directly contradictory to that furnished by the pyrolysis of solannelic acid, for an anhydride is

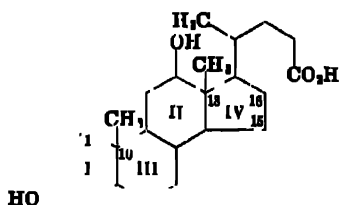
²³ Bernal, *Chemistry and Industry*, 51, 486 (1932)

²⁴ Wieland and Dane, *Z. physiol. Chem.*, 210, 369 (1932)

²⁵ Wieland and Weyland, *ibid.*, 110, 123 (1930).

formed in the one case and a ketonic ring is produced in the other. It is evident that the Blanc rule is not always a reliable guide, and from the information now available it appears that although the rule is probably valid for most open-chain acids and for diacids derived from cyclohexyl acetic and propionic acids, it is not applicable to compounds such as thilobilianic acid (or *allothilobilianic acid*) in which the carbon chain linking the two carboxyl groups also connects two ring systems. It appears that acids of this type can yield either anhydrides or ketones according to special, and as yet unrecognized, features of their structures. In contrast to thilobilianic acid and "isolithobilianic" acid, which easily form anhydrides, Vocke²⁶ found that two stereoisomeric perhydrodiphenic acids show little tendency to form anhydrides on pyrolysis but are very slowly converted into ketones.

That the Blanc rule failed to give accurate information regarding ring B destroyed all of the previous evidence that C is a five-membered ring, for this was derived from the behavior of acids of the same type as thilobilianic acid. The observation, on the other hand, did not of necessity invalidate the previous characterization of rings A and D. The situation having been clarified by discarding the previous misconceptions, it became possible to take full advantage of the new formula opportunely suggested by the English investigators. Although the chrysene structure of Rosenheim and King represented a most important advance it was in some respects contradicted by reliable evidence and required modification. It failed to show the connection known to exist between rings I (A) and III (B), but this was easily remedied by transposing these two rings (shown for desoxycholic acid). Wieland and Dane also pro-



Wieland and Dane (September, 1932)²⁶
Rosenheim and King (August, November, 1932)²⁷

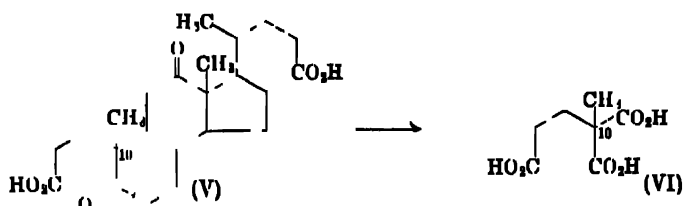
ferred to retain the cyclopentane structure for ring IV and to include a tertiarily bound methyl group between I and III. The formation of chrysene in the dehydrogenation might be explained as resulting from the pyrolytic rupture of ring IV and the closing of a new six-ring by the inclusion of the angular methyl group at C₁₀.

²⁶ Vocke, *Ann.*, 506, 1 (1934)

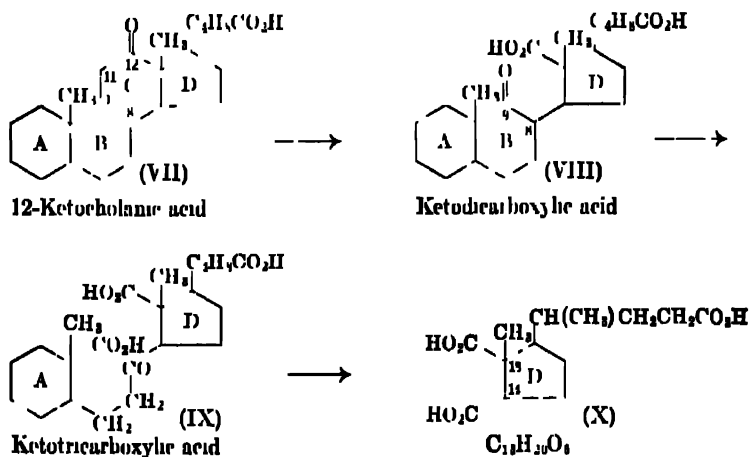
²⁷ Rosenheim and King, *Nature*, 130, 315 (1932); *Chemistry and Industry*, 51, 954 (1932).

the product (III) to the stepwise Grignard degradation. The ester of III gave with the phenyl Grignard reagent an unsaturated hydrocarbon, and this on oxidation with ozone yielded the nor-derivative of III. This established the presence of a methylene group at the original C₁-position, and a repetition of the degradation indicated a similar group at C₁ and gave the hisnor-acid IV. The methyl group obviously cannot be at C₁ and good evidence for the location at C₁₀ was found in the observation that the acid IV, like other tertiary carboxylic acids and unlike substances such as III, is esterified with considerable difficulty and loses carbon monoxide readily when warmed with concentrated sulfuric acid.

The location of a methyl group at the 10-position is in good accord with an earlier observation of Wieland and Voecke,³¹ who isolated α -methylglutaric- α -carboxylic acid (VI) as one of the oxidation products of the keto acid V ($C_{23}H_{34}O_6$) from pyrodesoxybilianic acid.



That the second methyl group is not situated at either of the bridge heads C₄ or C₉ is most clearly shown by Wieland's degradation of 12-ketocholanic acid (VII).³² Bromination at C₁₁ and hydrolysis gave the

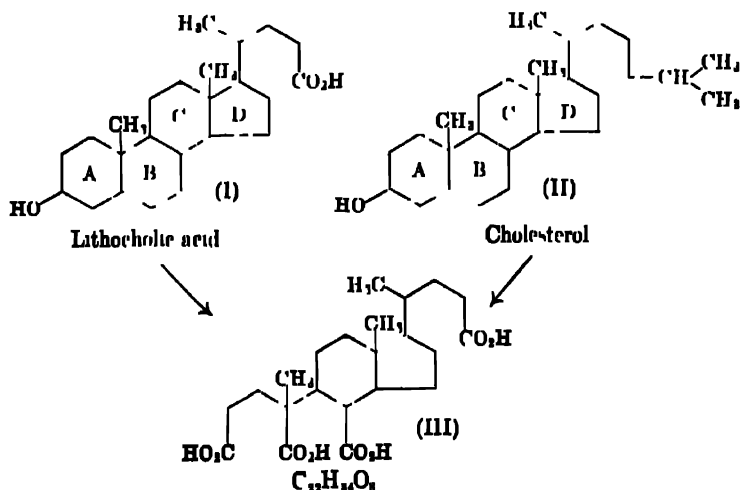
²¹ Wieland and Vocke, *Z. physiol. Chem.*, **177**, 68 (1928).

^a Wieland and Posternak, *ibid.*, 197, 17 (1981); Wieland and Dane, *ibid.*, 216, 91 (1983).

11-hydroxy-12-keto acid, and on oxidation this yielded the keto dicarboxylic acid, VIII. On bromination at C_{11} , followed again by hydrolysis and oxidation, ring B was opened at a hitherto inaccessible point with the formation of the acid IX, in which the original rings A and D are connected by remnants of the other rings originally present. This affords a clear demonstration of the relationship between rings B and C as well as showing that the second methyl group must be associated with D, the only ring remaining for discussion.

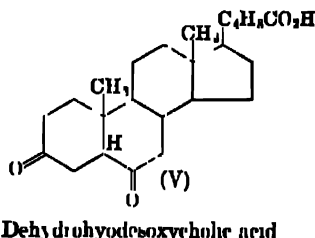
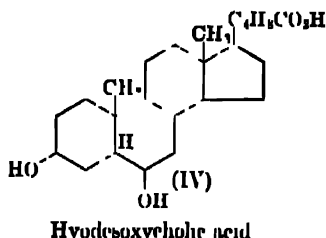
Further oxidation of the ketotricarboxylic acid (IX) gave the tri-carboxylic acid (X) which already had been obtained by the degradation of pyrodesoxybilianic acid. The observation³¹ that one of the three carboxyl groups of this acid can be esterified with diazomethane but not by the Fischer method is evidence that it is attached to a quaternary carbon atom, and this locates the methyl group at either C_{11} , or C_{14} . A more specific if more involved argument presented by Wieland and Danc³¹ from the stereochemical properties of the acid and its precursors places the methyl group at C_{11} , but a clearer decision between these two possibilities was achieved by applying the method of dehydrogenation, and this is true also of the problem of locating the position of attachment of the bile acid side chain. These points will be discussed below. In the work cited Wieland and Danc made the important observation that the acid X forms an anhydride only as the result of a rearrangement (to the *cis* acid), and consequently that it is a *trans* compound. This affords evidence of a *trans* linkage between the original rings C and D.

The Structure of Cholesterol. The relationship between the different



rings of the bile acids and the sterols was established definitely by the stepwise degradation of lithocholic acid (I) by the usual route: oxidation, pyrolysis to a ketone, ring cleavage.³⁴ The end product, III, was found to be identical with a tetracarboxylic acid which Windaus³⁵ had obtained previously from cholesterol by the cleavage of the oxygen-containing ring (A) with hypobromite, followed by the opening of the ring (B) containing the ethylenic linkage. The identity of the products correlates these rings with rings A and B of the bile acids.

It had long been suspected on biogenetic grounds that the hydroxyl group of cholesterol is located at C₃ rather than at C₄, as supposed earlier by Windaus, but it was not an easy matter to establish the point by correlating the sterols with the bile acids in this respect for the substances belong to two different stereochemical series. Windaus' important work on hydesoxycholic acid³⁶ from hog bile established the nature of the stereoisomerism and provided a means of passing from one series to the other. Without discussing the details of the proof, it may be said that hydesoxycholic acid (IV) was shown to be a 3,6-dihydroxycholanolic acid of the normal series. The diketo acid (V) obtained on careful oxidation



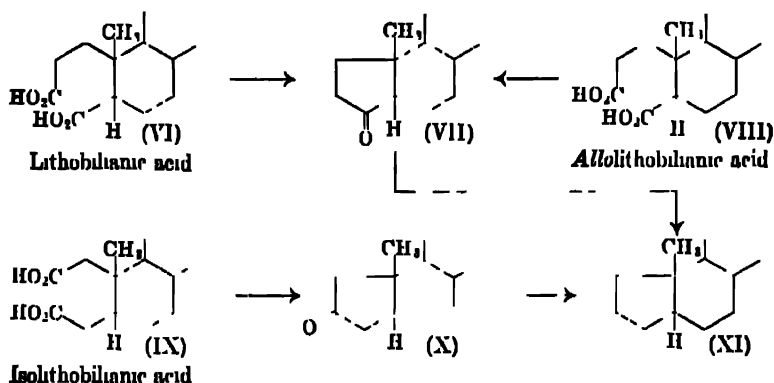
also belongs to the normal series, but the substance readily rearranges on treatment with acids or bases to a more stable stereoisomeride of the *allo*-series. The change clearly is due to an inversion at the asymmetric center (C₇) adjacent to the C₆-carbonyl group, for keto acids lacking this special feature of structure show no tendency to isomerize. By a careful selection of reactions and conditions, Windaus was able to convert hydesoxycholic acid into derivatives of both the normal and the *allo*-series, and from the behavior of these substances he was able to show that the cholanolic acid compounds have the configuration in rings A and B of *cis* decalin while the *allo*-compounds are of the *trans* type, as might be expected from the greater stability of dehydrohydesoxy*allo*cholic acid, as compared with its isomer. The proof is as follows. Since lithobilanic

³⁴ Wieland, Dane and Scholz, *Z. physiol. Chem.*, **211**, 261 (1932). A further demonstration of the point at issue was made by Stange, *ibid.*, **220**, 34 (1933).

³⁵ Windaus, *Ber.*, **42**, 3770 (1909).

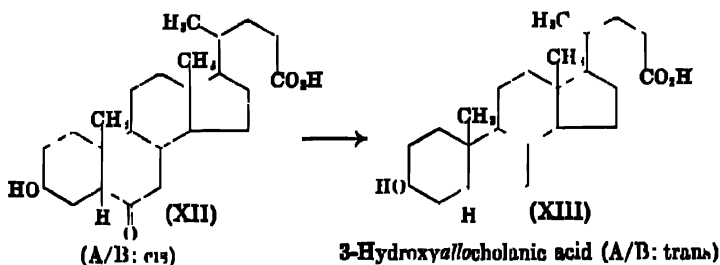
³⁶ Windaus, *Ann.*, **447**, 233 (1926).

acid (VI) and *allolithobilianic* acid (VIII) both yield the same keto acid (VII) on pyrolysis, one or the other of these acids must suffer a rearrangement in the process through an inversion at the position (C_6) adjacent to the carboxyl group. The pyroacid VII must have the more easily



formed *cis* configuration, and the *desoxyacid* (XI) obtained on reduction may be regarded as a *cis* compound. Isolithobilianic acid (IX) and its *allo*-stereoisomeride (Staden's acid) are not of the type susceptible to partial racemization (no α -C*) and they yield stereoisomeric pyroacids in which the original configurations are retained. The pyroacid from isolithobilianic acid (IX) was found to give the *cis* desoxyacid (XI) on reduction, and, since the lithobilianic acids are of the normal cholan acid series, cholan acid is a *cis* compound. *Allocholan* acid and dihydrocholesterol are of the *trans* decalin type.

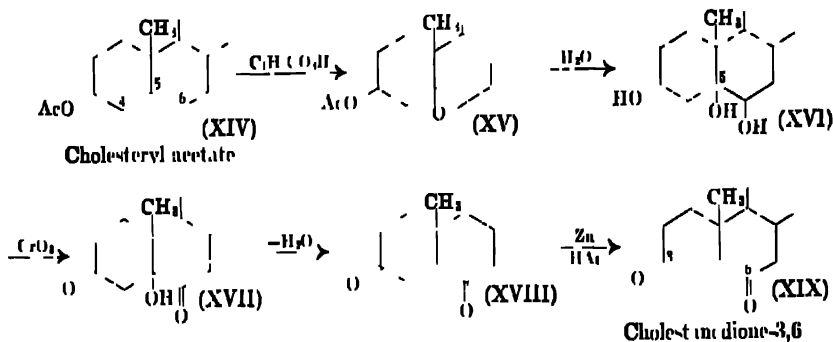
Following these discoveries, Wieland and Dane³⁷ were able to correlate a 3-hydroxycholan acid with cholesterol as follows. Hyodesoxycholic acid was converted by partial oxidation into 3-hydroxy-6-keto-cholan acid (XII). On reduction by the Wolff-Kishner method this suffered partial allomerization, and 3-hydroxy*allo*cholan acid (XIII) was isolated from the reaction mixture. The substance is isomeric with litho-



³⁷ Wieland and Dane, *Z. physiol. Chem.* 212, 41 (1932)

cholic acid, and it was found to be identical with a substance which had been obtained previously by Windaus³⁸ from cholesterol by protecting the hydroxyl group, saturating the double bond, and shortening the side chain by oxidation, as follows: cholesterol \longrightarrow cholesteryl chloride \longrightarrow cholestyl chloride \longrightarrow 3-chloroallocholanolic acid \longrightarrow 3-hydroxyallocholanolic acid. The identity of the products proved that the hydroxyl group of cholesterol occupies the characteristic 3-position.

To complete the evidence it is necessary to show that the double bond of cholesterol is in the β,γ -position with respect to the hydroxyl group rather than in the α,β -position, as in the case of cholestenone. Conclusive evidence on this point is found in the following series of transformations, starting with cholesteryl acetate (XIV). This can be converted into a triol (XVI) either by direct oxidation³⁹ or through the oxide (XV), which is obtained with the use of perbenzoic acid:⁴⁰



On oxidation of the triol (XVI) only two of the alcoholic groups are attacked, indicating that the remaining group is in a tertiary location, as at C₃. This hydroxyl is easily eliminated from the diene-ol (XVII) and the unsaturated linkage of XVIII can be reduced chemically, giving the saturated diketone (XIX). The relative positions of the carbonyl groups in cholestanedione are fixed by the fact that the substance condenses with hydrazine to form a pyridazine derivative,⁴¹ a property characteristic of 1,3- and 1,4-diketones. From stereochemical considerations it is probable that the cyclic product is constituted as in XXa or XXb,⁴² but the structure is not material to the argument. The significant point is that the carbonyl compound is shown not to be an α -diketone, as would be expected if the double bond of cholesterol were located at C₁-C₆. Since

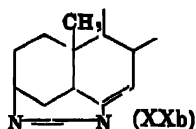
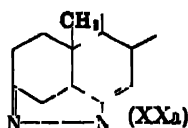
³⁸ Windaus and Howfeld, *Z. physiol. Chem.*, **145**, 177 (1925)

³⁹ Windaus and Kirchner, *Ber.*, **53**, 614 (1920)

⁴⁰ Westphalen, *Ber.*, **48**, 1064 (1915)

⁴¹ Windaus, *ibid.*, **39**, 2249 (1906)

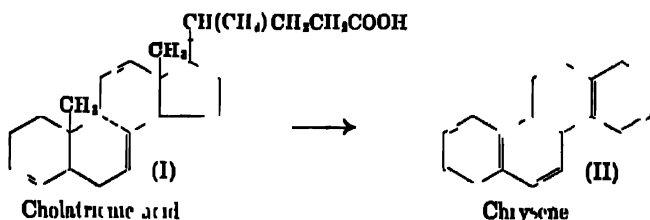
⁴² Farnholz, *Ann.*, **508**, 215 (1934); Windaus, Inhoffen and v. Reichel, *ibid.*, **510**, 251 (1934)



a β -diketonic structure is out of the question, the end product is definitely identified as a γ -diketone and the original double bond must extend into ring B, as at C_5 - C_6 .

Dehydrogenation. Although the method of dehydrogenation with selenium discovered by Diels has become a most valuable tool for the investigation of various natural products, it was not at first a simple matter to evaluate properly the evidence from this source regarding the structures of the sterols and bile acids. Diels⁴³ had reported the formation of chrysene in the dehydrogenation of cholic acid with selenium at 360° , and the observation had been of influence in the revision of the early formulas. Ruzicka⁴⁴ repeated Diels' experiments and was at first unable to detect any chrysene in the mixtures of aromatic hydrocarbons obtained by what appeared to be essentially the same method, but after Diels⁴⁵ had advanced further evidence in support of his identification of the hydrocarbon in question, and after other workers⁴⁶ had reported the formation of chrysene, Ruzicka⁴⁷ finally traced the difference between his earlier results and those of Diels to a difference in temperature. On dehydrogenating cholic acid or cholatrienic acid at 420° he obtained both chrysene and picene, and at a lower temperature (360°), according to his observations, the presence of chrysene is largely obscured by the formation of another hydrocarbon ($C_{21}H_{18}$, m.p. 275°) which is not stable at the higher temperature.

According to the accepted structures, the formation of chrysene results from a severing of the bond holding the acid side chain, the rupture of ring D, and the incorporation of the methyl group at C_{11} into a new, six-



⁴³ Diels, Gadler and Körding, *Ann.*, **459**, 1 (1927); Diels and Karstens, *ibid.*, **478**, 129 (1930).

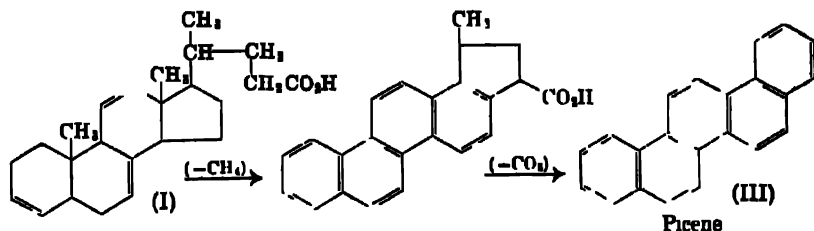
⁴⁴ Ruzicka and Thomann, *Helv. Chim. Acta*, **16**, 216 (1933); Ruzicka, Goldberg and Thomann, *ibid.*, **16**, 512 (1933).

⁴⁵ Diels, *Ber.*, **66**, 487, 1122 (1933).

⁴⁶ Raudnitz, Petró and Stadler, *ibid.*, **66**, 879 (1933); Cook and Howett, *J. Chem. Soc.*, 1008 (1933).

⁴⁷ Ruzicka, Thomann, Brandenberger, Furter and Goldberg, *Helv. Chim. Acta*, **17**, 300 (1934).

membered ring (I \longrightarrow II). The formation of picene (III) is perhaps the result of a similar enlargement of ring D, the closing of a new five-membered ring from the interaction with the side chain, and the rearrangement of the methylcyclopentene ring to a six-membered aromatic ring.



It is clear that reactions involving such extensive rearrangements give only a rough indication of the character of the original ring system.

From a study of the behavior of alkylated hydrindenes when heated with selenium or with palladium charcoal at a very high temperature (450°), Ruzicka and Peyer⁴⁸ concluded that the aromatization of an alkylated five-ring often occurs under these conditions but that a rational formulation is not always possible. These investigators made the interesting observation that indene, when heated with selenium or palladium charcoal at 350° , are partly destroyed and partly converted into hydrindenes. The hydrogenation of an unsaturated five-ring, particularly in the presence of selenium, is a remarkable process. A hydrogenating and isomerizing action of selenium has been observed by Doré and Petrow⁴⁹ who found that at 230° cholesterol is converted in part into cholestanone, and, in smaller amounts, into cholestanol and cholestenone. That selenium exerts only a weak dehydrogenating action is also indicated by the fact that cyclohexane rings containing obstructing, tertiarily bound groups, are not always capable of being aromatized.⁵⁰

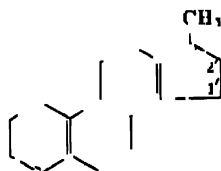
The Diels Hydrocarbon. Of much more importance to the structural problem was the isolation by Diels, Gädke and Kording⁴³ in 1927 of a dehydrogenation product melting at $124\text{--}125^\circ$ and originally assigned the empirical formula $\text{C}_{18}\text{H}_{16}$. This was obtained along with a second hydrocarbon ($\text{C}_{22}\text{H}_{24}$) by the action of selenium on cholesteryl chloride or cholesterol, and it has been the subject of much active investigation and of much debate. In the first study of the Diels hydrocarbon ($\text{C}_{18}\text{H}_{16}$) the substance was purified, analyzed, and characterized with great care,

⁴⁸ Ruzicka and Peyer, *Helv. Chim. Acta*, **18**, 876 (1935)

⁴⁹ Doré and Petrow, *J. Chem. Soc.*, 1391 (1935) See also Yokoyama and Kotaka, *Bull. Chem. Soc. Japan*, **10**, 138 (1935)

⁵⁰ Clemo and Dickenson, *J. Chem. Soc.*, 745 (1935), Cook, Danu, Hewett, Hall, Maynesord and Roe, *ibid.*, 1319 (1935).

and a particularly distinctive reaction was found in the conversion of the hydrocarbon into a nitroso compound ($C_{18}H_{17}O_2N$) with oxides of nitrogen. Diels and Karstens⁴³ obtained the same hydrocarbon (and the hydrocarbon $C_{22}H_{34}$) in the dehydrogenation of ergosterol, and Ruzicka and his collaborators⁴⁴ reported its isolation, together with three other hydrocarbons, from the mixtures resulting from the action of selenium on cholic acid, and pointed out the difficulty in distinguishing between the formulas $C_{18}H_{18}$ and $C_{17}H_{14}$ from the available analyses. In May 1933, Rosenheim and King⁵¹ reported the results of a study of the ultra-violet-absorption spectrum of the Diels hydrocarbon from which they were led to suggest tentatively the structure of 3'-methyl-1,2-cyclopentenophenanthrene (or γ -methylecyclopentenophenanthrene):

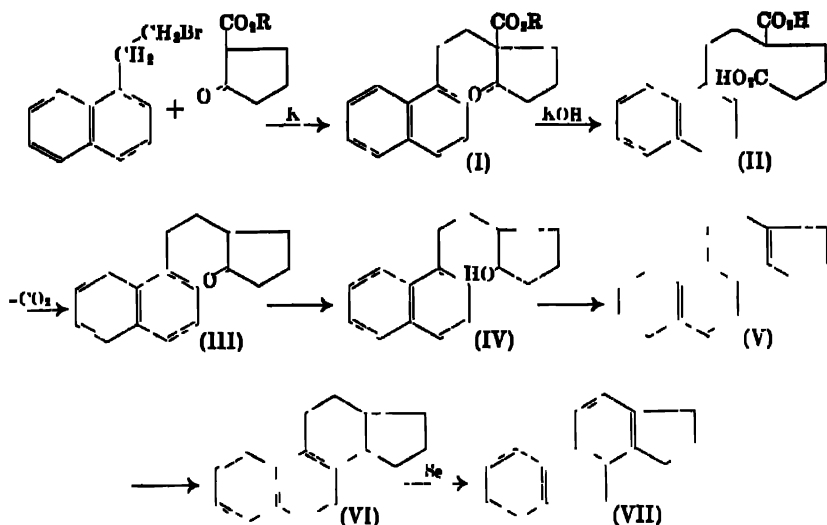


Essentially the same idea had occurred to Ruzicka, and possibly to other investigators, at about the same time and there followed in rapid succession a number of attempts to settle the matter by synthesis. This line of attack was particularly inviting because elaborate methods for the synthesis of phenanthrene derivatives had been made available by Haworth, Bardhan and Sengupta, and Bogert and co-workers in the course of the investigation of the degradation products of the resin acids. Ruzicka and his co-workers⁵² soon reported the synthesis of 1,2-cyclopentenophenanthrene, and of the 1'- and 2'-methyl derivatives, using the method of Bardhan and Sengupta, and Kon⁵¹ shortly afterwards described the preparation of the first of these hydrocarbons by the same method. β -(1-Naphthyl)-ethyl bromide condensed fairly well (65% yield) with ethyl cyclopentanone-2-carboxylate to give the β -keto ester I, but the decarboxylation of this substance proceeded so poorly that the ketone III was best obtained by way of the dibasic acid II. Kon did not isolate the unsaturated hydrocarbon V, but heated the carbinol IV with phosphorus pentoxide and obtained VI directly. Treatment with selenium gave the desired hydrocarbon VII, it being known already that the five-membered ring is not dehydrogenated by the action of selenium. Ruzicka used a shorter method, finding that the keto ester I was converted directly into the final hydrocarbon VII on being heated with strong sulfuric acid.

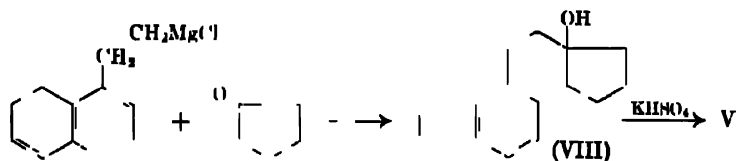
⁵¹ Rosenheim and King, *Chemistry and Industry*, 52, 299 (1933)

⁵² Ruzicka, Ehmann, Goldberg and Hosh, *Helv Chim Acta*, 16, 833 (1933)

⁵³ Kon, *J Chem Soc*, 1081 (1933).



Cook and Hewett,⁵⁴ who had become interested in the problem because of the possibility that some of the dehydrogenation products from the sterols might have carcinogenic properties, synthesized cyclopentenophenanthrene by a method of the general type described by Perlman, Davidson and Bogert a few months earlier, the first step consisting in

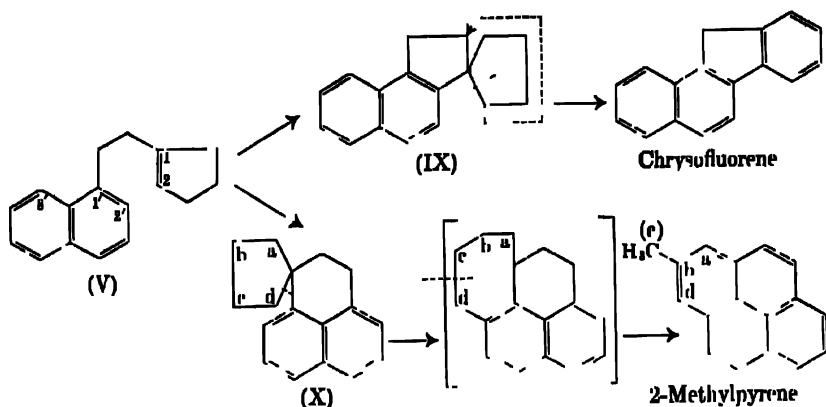


the condensation of β -(1-naphthyl)-ethyl magnesium chloride with cyclopentanone. The unsaturated hydrocarbon V is an intermediate in both syntheses, for it can be obtained by the controlled dehydration of both the carbinols IV and VIII. For the preparation of cyclopentenophenanthrene (VII), Cook and Hewett found the isolation of intermediate hydrocarbons unnecessary, for when the carbinol VIII is heated with a mixture of sulfuric and acetic acids it yields the aromatic hydrocarbon VII directly, dehydration and cyclization being followed by a dehydrogenation at the expense of the sulfuric acid.

The cyclization of the unsaturated hydrocarbon V with aluminum chloride or stannic chloride was studied more extensively by Cook and

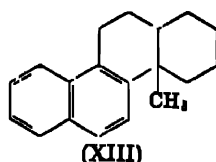
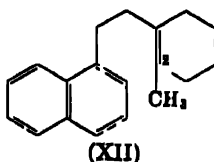
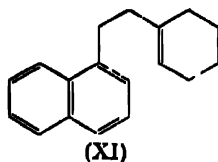
⁵⁴ Cook and Hewett, *Chemistry and Industry*, 52, 451, 603 (1933). *J. Chem. Soc.*, 1008 (1933)

Hewett in a later investigation,⁵⁵ and they were able to establish the nature of two by-products which accompany the hydrocarbon VI. In addition to the normal intramolecular condensation between positions C₂ and C₂', condensation also appears to take place between positions C₁ and C₂', giving the spiran IX, and between C₁ and C₆', with the formation of X.



On dehydrogenating these hydrocarbons with selenium at a high temperature the five-membered spiran rings undergo rearrangement and aromatization, and the final products are chrysofluorene and 2-methylpyrene.⁵⁶ Possible mechanisms for the production of the new aromatic rings are suggested in the formulas. The transformations are comparable with Clemo and Ormston's⁵⁷ conversion of cyclohexanespirocyclopentane into naphthalene by treatment with selenium.

The formation of spirans materially diminishes the yield of the desired product, but in later work Cook⁵⁸ found that the tendency to form spirans is greatly diminished if a methyl group is introduced on the second carbon atom (C₂) of the ethylenic linkage. In the synthesis of chrysene, for example, whereas the unsaturated hydrocarbon XI yields chiefly spirans on cyclization, the methyl derivative XII is converted in good



⁵⁵ Cook and Hewett, *J. Chem. Soc.*, 365 (1934). See also A. Cohen, Cook and Hewett, *ibid.*, 1633 (1935).

⁵⁶ Regarding the identification of this hydrocarbon see Barry, Cook, *et al.*, *Proc. Roy. Soc. (London)*, B117, 321, footnote (1935).

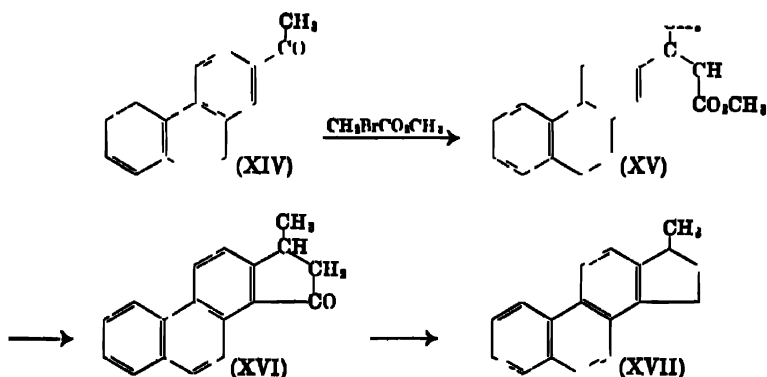
⁵⁷ Clemo and Ormston, *J. Chem. Soc.*, 352 (1933).

⁵⁸ Cook and co-workers, *ibid.*, 652, 1727 (1934); 667 (1935).

yield into methyloctahydrochrysene (XIII), from which chrysene can be obtained by dehydrogenation with selenium (but not with platinum black).

There was at first some difference in opinion as to the relationship of the Diels hydrocarbon to the synthetic preparations. Of the three hydrocarbons synthesized by Ruzicka,⁵² the 1'- and 2'-methyl derivatives were clearly quite different, but 1,2-cyclopentenophenanthrene (m.p. 135°) bore some resemblance to the Diels hydrocarbon (m.p. 125°). The difference in melting point indicated that the two substances either were not identical or that, contrary to the evidence from the constancy of the melting points, they were not equally pure. It was not easy to make a decision between these two possibilities because mixtures of the two samples melted at intermediate temperatures, and this was true also of the molecular compounds with picric acid, trinitrobenzene, and trinitrotoluene. Ruzicka was inclined to consider the substances identical, but he deferred judgment in the matter, and Kon,⁵³ whose observations agreed exactly with those of Ruzicka, took the same position. Cook and Hewett,⁵⁴ however, were definitely of the opinion that their cyclopentenophenanthrene (m.p. 135°) was identical with the Diels hydrocarbon from cholesterol and that the latter substance had never been obtained in a completely pure condition.

Bergmann and Hillemann⁵⁵ were the first to synthesize 3'-methylcyclopentenophenanthrene, but the results were not decisive. The substance melted within one degree of the melting point of the Diels hydrocarbon, but mixtures showed depressions of 1-5°. The starting point for the synthesis was 2-acetylphenanthrene (XIV). The unsaturated acid

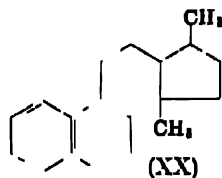
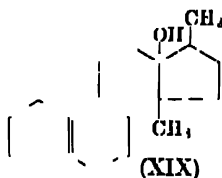
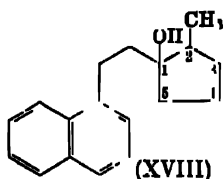


obtained on hydrolysis of the product of the Reformatsky reaction (XV) was reduced and converted through the acid chloride into the cyclic ketone,

⁵⁵ E. Bergmann and Hillemann, *Ber.*, 66, 1802 (1933).

from which the final product (XVII) was obtained by the Clemmensen method.

Another synthesis of 3'-methylcyclopentenophenanthrene was carried out in 1934 by Harper, Kon and F. C. J. Ruzicka,⁶⁰ using the Perlman-Davidson-Bogert method. The carbinol (XVIII) from β -(1-naphthyl)-



ethyl magnesium bromide and 2-methylcyclopentanone proved to be of no value for the purpose at hand because the double bond introduced on dehydration appeared at the 1,2- rather than the 1,5-position, and the methyl group was eliminated in the final dehydrogenation. In order to overcome this difficulty 2,5-dimethylcyclopentanone was employed as the starting material and the carbinol XIX was subjected to cyclodehydration (with phosphorus pentoxide at 140°). The hydrocarbon XX was obtained in good yield in this way and on treatment of the substance with selenium the tertiarily bound methyl group was eliminated and 3'-methylcyclopentenophenanthrene was isolated in a pure condition (m.p. 125-6°) from the resulting mixture by crystallizing the trinitrobenzene derivative. The melting point was identical with that of a sample of the Diels hydrocarbon and there was no depression in the melting points of mixtures. It had become quite apparent by this time, however, that the method of mixed melting point determinations cannot be relied upon in this series of compounds. No depression was noted by the above investigators with mixtures of cyclopentenophenanthrene and its 3'-methyl derivative, and a similar observation has been reported by Jacobs and Fleck⁶¹ for mixtures of the Diels hydrocarbon with a substance having the composition of a dimethylphenanthrene. Comparison by way of the absorption spectra is not valid with such closely related substances, for the differences between homologues is too slight to be significant. Bernal made X-ray and crystallographic comparisons of the substances prepared by various investigators and expressed the opinion⁶² that the Diels hydrocarbon is identical with the samples of 3'-methylcyclopentenophenanthrene prepared by Bergmann and Hillemann and by Harper, Kon, and F. C. J.

⁶⁰ Harper, Kon and F. C. J. Ruzicka, *J. Chem. Soc.*, 124 (1934)

⁶¹ Jacobs and Fleck, *J. Biol. Chem.*, 97, 57 (1932).

⁶² See Bernal and Crowfoot, *J. Chem. Soc.*, 93 (1933)

Ruzicka, but not with the unmethylated hydrocarbon as claimed by Cook and Hewett.

It became highly desirable to test these conclusions further and to apply chemical methods to the question of identity, but the chemical characterization of the Diels hydrocarbon presented more difficulties than would have been anticipated. Various investigators have attempted without success to convert the hydrocarbon into a quinone, and it has been suggested that the five-membered ring is attacked by oxidizing agents before the phenanthrene nucleus. In his original paper of 1927 Diels had recommended as a means of identification the conversion of the hydrocarbon into a characteristic nitroso compound, and he later noted⁶³ that a sample of cyclopentenophenanthrene supplied by Cook and Hewett remained unchanged when treated with oxides of nitrogen in the same manner. Synthetic samples of 3'-methylcyclopentenophenanthrene do not appear to have been tested in this way until 1935, when Hillemann⁶⁴ repeated his early synthesis with Bergmann. The highly purified material melted at 125-126° and showed the same melting point when mixed with the "sterol-C₁₄H₁₆." Hillemann succeeded in converting both the synthetic hydrocarbon and that obtained by dehydrogenation into the characteristic nitroso compound, m.p. 238-239° (corr.) of Diels, and this affords a convincing indication of the identity of the substances. Hillemann also isolated mellophanic acid as an oxidation product of his synthetic hydrocarbon, which proves that the cyclopentene ring actually is attached to the phenanthrene nucleus in the 1,2-position and that it contains the methyl group.

The structure of Diels' nitroso compound is not yet known, and indeed the composition is still subject to some uncertainty.⁶⁴ The yield appears to be very poor, and so uncertain is the reaction by which it is formed that Diels and Rickert⁶⁵ reported their failure to obtain the nitroso compound from a sample of synthetic hydrocarbon supplied by Kon. Diels and Rickert, however, discovered another characteristic derivative in a tribromo compound melting at 235°, and they found that samples prepared from the sterol C₁₄H₁₆ and from the material synthesized by the procedure of Harper, Kon and F. C. J. Ruzicka are identical. This conclusion has been further confirmed by Gamble, Kon and Saunders.⁶⁶

On the basis of these comparisons the structure of the Diels hydrocarbon can be regarded as firmly established. Although the yield in the dehydrogenation is very poor and the conditions are rather drastic, the formation of this aromatic hydrocarbon is of such regular occurrence that

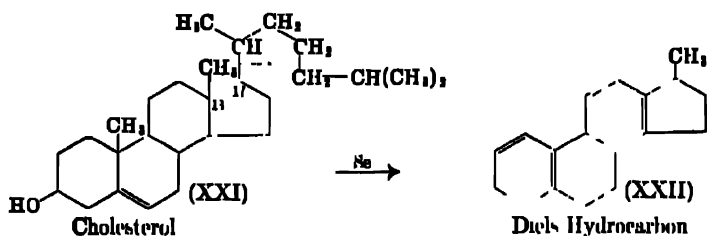
⁶³ Diels and Klare, *Ber.*, **67**, 113 (1934)

⁶⁴ Hillemann, *ibid.*, **68**, 102 (1935)

⁶⁵ Diels and Rickert, *ibid.*, **68**, 287, 325 (1935)

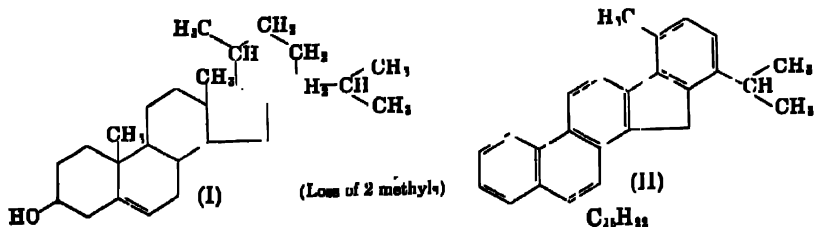
⁶⁶ Gamble, Kon and Saunders, *J. Chem. Soc.*, 611 (1935)

it can be accepted as affording reliable evidence of the aetiocholane structure, particularly since it is known that although five-membered rings can be rearranged to six-membered rings under the conditions of the dehydrogenation they do not appear to be formed in such a process. The four rings of the cholane system undoubtedly correspond with those of 3'-methylcyclopentenophenanthrene, and the appearance of the methyl group in the five-ring finds a ready interpretation in the wandering of the methyl radical at C_{15} to the position at C_{17} , varated in the pyrolytic cleavage of the branched side chain: XXI \longrightarrow XXII. Cohen, Cook



and Hewett⁶⁷ have pointed out that the methyl migration must be concomitant with the elimination of the side chain and they have suggested that the process is a special case of the Wagner-Meerwein rearrangement.

The Second Diels Hydrocarbon. The nature of another aromatic hydrocarbon isolated by Diels, Gädke and Kording from the mixture obtained in the dehydrogenation of cholesterol is not yet clear. According to Diels the substance melts at 220° , it has the formula $C_{24}H_{14}$, and it yields a ketone on oxidation, indicating the presence of a five-membered ring flanked by phenyl groups. Other investigators who have isolated the hydrocarbon are in essential agreement with regard to the properties and composition, although Cook and co-workers⁶⁸ have contended that the formulas $C_{26}H_{22}$ and $C_{26}H_{24}$ are not excluded. The revised melting point is $225-226^\circ$, corr. Following the suggestion of Rosenheim and King⁵¹ that the substance may have arisen by the transformation I \longrightarrow II,



⁶⁷ A. Cohen, Cook and Hewett, *J. Chem. Soc.*, 445 (1938). See also, E. Bergmann, *Chemistry and Industry*, 54, 175 (1935).

⁶⁸ Cook, Hewett, Maynard and Roe, *Chemistry and Industry*, 53, 560 (1934).

Cook and co-workers^{68,69} synthesized the hydrocarbon indicated (modified Perlman-Davidson-Bogert synthesis) and compared it with the Diels product. The two substances were not identical, but the incidence in the absorption spectra was so great as to suggest a similar type of structure.⁷⁰ The English investigators have suggested that one of the tertiary methyls may wander into a ring in the course of the dehydrogenation and that the Diels compound may be a higher homologue ($C_{26}H_{44}$) of the synthetic compound II.

Although Diels claimed repeatedly⁷¹ that ergosterol yields the identical hydrocarbon (m.p. 225°), this point has been contested by Ruzicka,⁷² who has presented evidence to the effect that cholesterol (C_{27}) yields $C_{25}H_{40}$ (225°), that ergosterol (C_{28}) yields $C_{26}H_{42}$ (214°), and that a mixture of the C_{27} -sterols, stigmasterol and sitosterol, gives the next higher homologue, $C_{27}H_{44}$ (202°). The question probably is not one of great significance to sterol chemistry except in connection with the mechanism of dehydrogenation, but it would be a matter of great interest if any of these polynuclear hydrocarbons should be found to possess carcinogenic activity.

The Position of the Side Chain. One of the most important dehydrogenation products obtained from the sterols or bile acids is methylcholanthrene, the substance described in the previous chapter as having marked cancer-producing properties. This was not obtained by the direct dehydrogenation of a natural product, but by the following degradation of desoxycholic acid. 12-Ketocholanic acid, obtained by the partial reduction of dehydrodesoxycholic acid by the Clemmensen method, was found by Wieland⁷³ to undergo intramolecular condensation with loss of carbon dioxide and water when heated for several hours at 330° . The carbonyl group at C_{12} condenses with the α -methylene group of the side chain after the manner of the Perkin reaction and the unsaturated acid then becomes decarboxylated, giving the beautifully crystalline dehydronorcholene. That no molecular rearrangement occurs in the course of the pyrolytic reaction was proved by Cook and Haslewood.⁷⁴ The keto acid resulting from the oxidation⁷⁵ of dehydronorcholene was reduced by the Wolff-Kishner method and the product was found to be identical with norcholanic acid, previously obtained by Wieland, Schlichting and Jacobi¹⁵ by the

⁶⁸ Cook, Hewett, Maynard and Roe, *J. Chem. Soc.*, 1727 (1934).

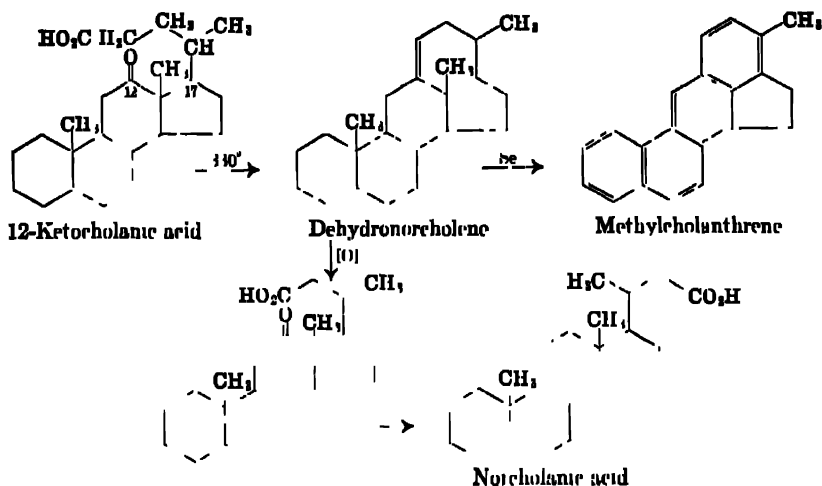
⁶⁹ This conclusion has been confirmed by further comparison studies of Cook, Daniel, Hewett, Iball, Maynard and Roe, *ibid.*, 1319 (1935).

⁷⁰ Diels and Karstens, *Ann.*, 478, 120 (1930); Diels, *Ber.*, 66, 1123 (1933); Diels and Klare, *ibid.*, 67, 113 (1934).

⁷¹ Ruzicka, Goldberg and Thomann, *Helv. Chim. Acta*, 16, 812 (1933); Ruzicka, Thomann, Brandenburg, Farber and Goldberg, *ibid.*, 17, 200 (1934); Ruzicka and Goldberg, *ibid.*, 18, 434 (1935).

⁷² Wieland, Schlichting and Wiedersheim, *Z. physiol. Chem.*, 150, 273 (1925); Wieland and Wiedersheim, *ibid.*, 186, 229 (1930).

⁷³ Cook and Haslewood, *J. Chem. Soc.*, 428 (1934).



Grignard degradation of cholic acid. Methylcholanthrene is obtained from the unsaturated hydrocarbon by the action of selenium,⁷⁶ and the structure of the yellow, aromatic hydrocarbon has been established both by oxidative degradation (p. 86) and by synthesis (p. 105). Since the only carbon atoms lost in the dehydrogenation are those at the two bridge heads, and since no rearrangements or migrations are involved, this series of reactions affords very reliable evidence of the cholane ring system. Still more important is the proof that the bile acid side chain is attached to ring D at the C₁₇-position, for the only other information on this point is that the calculated molecular dimensions agree most closely with the X-ray measurements if the side chain is placed at C₁₇. On stereochemical grounds the cyclization of 12-ketocholic acid is consistent only with this point of attachment, and the above formulation is the only way of accounting for the formation of a new ring capable of becoming aromatic.

One other inference as to structure may be made from the observation of Butenandt⁷⁶ that Wieland's aetiobilanic acid yields 1,2-dimethylphenanthrene on dehydrogenation with selenium. In point of time this was the first clear proof that the cholane structure contains a hydrogenated phenanthrene nucleus. The reaction is readily understandable when the



Aetiobilanic acid

⁷⁶ Wieland and Dane, *Z. physiol. Chem.*, **219**, 210 (1933)

⁷⁷ Butenandt, Weidlich and H. Thompson, *Ber.*, **66**, 801 (1933)

acid oxidation product in this case contained one methylene group more in the side chain than VII and it was identical with the material obtained by Wieland from hyodesoxycholic acid (page 156). On shortening the acid chain of IX by degradation according to Wieland, Schlichting and Jacobi, an acid was obtained identical with that (VII) from stigmasterol. This observation proved not only that the hydroxyl group of stigmasterol occupies a position corresponding to that of cholesterol but also that the configuration of the carbon atom (C_3) carrying the hydroxyl group is the same in each case. Compounds VI-IX all belong to the stereochemical series of dihydrocholesterol both with regard to the configuration at C_3 (β -type) and the relationship between rings A and B (*allo*-series). The reduced sterols of the β -type are precipitated by digitonin.

That the nuclear double bond of stigmasterol occupies the 5,6-position was established by applying methods developed at the Göttingen laboratory in the case of cholesterol (page 157). Fernholz⁸³ converted stigmasterol through the oxide-acetate and triol into stigmastanedione-3,6 and found that the diketone forms a pyridazine derivative with hydrazine. Following the reasoning outlined above, this fixes the point at issue and it is clear that the structure of the phytosterol is firmly grounded in all details.

Ergosterol. Ergosterol was first isolated from ergot and it is now prepared in considerable quantity from yeast. The chemistry of the sterol has been explored with great interest on account of its relationship to vitamin D, but peculiar difficulties have been encountered in the work on the sterol and its many transformation products and there are still some points of uncertainty with regard to the structures.

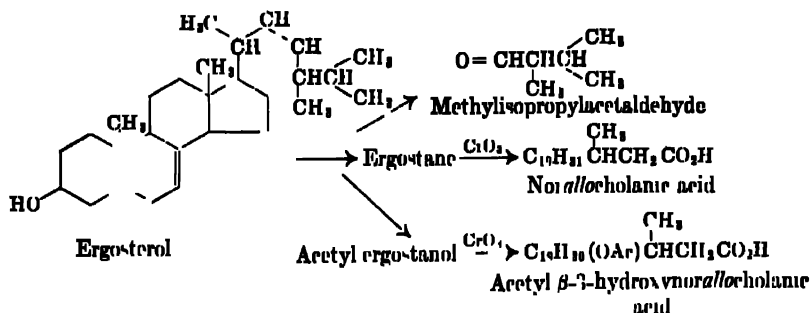
Following the establishment of the composition $C_{28}H_{44}O$ for ergosterol by Windaus⁸⁴ in 1932, rapid progress was made in determining the character of the carbon framework by various workers at the Göttingen laboratory. Reindel and Kipphan,⁸⁵ and later Guiteras,⁸⁶ obtained evidence regarding the structure of the side chain and the location of the external double linkage by isolating methylisopropylacetaldehyde as a product of ozonization. Chuang⁸¹ oxidized the saturated hydrocarbon ergostane and isolated an acid which was identified as norallocholic acid, thus correlating the ring system and the first four carbon atoms of the side chain with cholesterol. Fernholz and Chakravorty,⁸² by the isolation of an acid oxidation product of acetyl ergostanol identical with that obtained directly from stigmasterol and indirectly from cholesterol (page 170),

⁸³ Fernholz, *Ann.*, 506, 215 (1934).

⁸⁴ Windaus and Lüttringhaus, *Nachr. Ges. Wiss., Göttingen*, 4 (1932).

⁸⁵ Reindel and Kipphan, *Ann.*, 463, 181 (1932).

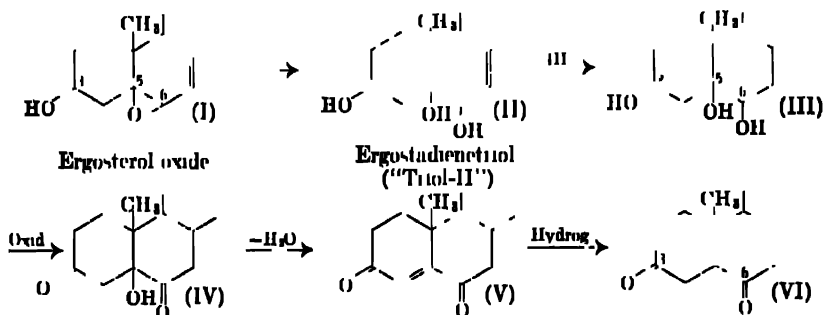
⁸⁶ Guiteras, *ibid.*, 494, 116 (1932).



proved that the hydroxyl group occupies the 3-position and that the configuration is that of dihydrocholesterol (β -type, precipitated by digitonin).

The most difficult part of the problem is in locating the two nuclear double linkages. A suggestion that they are both contained in the same nucleus was afforded by the observation that ergosterol on oxidation with nitric acid yields an aromatic acid⁴⁷ (compare the oxidation of abietic acid, page 60). The reaction is abnormal, however, for the oxidation product is a methylbenzene tetracarboxylic acid, and a methyl group must migrate in the course of its formation. The point is more securely established by evidence to be presented below.

That one of the nuclear double bonds is located at the "cholesterol" position (C_7-C_8) was established beyond dispute by Windaus, Inhoffen and v. Reichel.⁴⁸ Ergosterol oxide (I), which can be obtained (as the benzoate) by the addition of perbenzoic acid to one of the three double bonds of the sterol, yields on hydrolysis ergostadienetriol, II (called the "triol-II" in order to distinguish it from an isomer to be described below).⁴⁹ One of the new hydroxyl groups is secondary while the other is tertiary, for the triol forms a diacetyl derivative. There is one ethylenic linkage in the ring system and one in the side chain, and on hydrogenation⁵⁰ (of



⁴⁷ Inhoffen, *Ann.*, **494**, 123 (1932), see also Reindel and Niederlander, *ibid.*, **482**, 264 (1930)

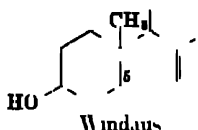
⁴⁸ Windaus, Inhoffen and v. Reichel, *ibid.*, **510**, 248 (1931)

⁴⁹ Windaus and Lüttringhaus, *ibid.*, **481**, 127 (1930)

⁵⁰ Heilbron, Morrison and J. C. W. Simpson, *J. Chem. Soc.*, 302 (1933)

the diacetyl derivative) the substance can be converted into the saturated triol III. According to the observations of Windaus, Inhoffen and v. Reichel, the latter compound closely parallels in its reactions the corresponding triol of the cholesterol series, for which the structure of cholestanetriol-3,5,6 had been firmly established by Windaus (page 157). Like this compound, III yielded in succession a dione-ol (IV), an ergostenedione (V), and an ergostadione (VI) which forms a pyridazine derivative with hydrazine. As in the case of cholesterol, this proves that perbenzoic acid combines with an ethylenic linkage at the 5,6-position. Independent evidence in support of this point has been advanced by Heilbron.⁹¹

The location of the second nuclear double bond is the only point in the structure of ergosterol about which there has been any difference of opinion. According to the available evidence the only locations possible are C₇-C₈ and C₈-C₉, as indicated by the formulas for ergosterol sup-



ported respectively by Windaus and by Heilbron. The distinction is a subtle one and with a compound as labile and as sensitive to change as ergosterol the matter is not easily decided. Such direct evidence as is available definitely favors the Windaus formula, according to which the unsaturated linkages occupy positions of conjugation. Analyses of the absorption spectrum (maximum at 280 mμ), of molecular refraction data,⁹² and of X-ray measurements⁹³ all point in this direction, and the fact that the sterol can be reduced with sodium and amyl alcohol⁹⁴ indicates the presence of a conjugated system. Of particular significance is the formation of a characteristic addition product when ergosterol is heated with maleic anhydride at a temperature of 135°.⁹⁵ This shows for one thing that both of the double bonds must be located in the same ring, and the observation is most easily interpreted in terms of a simple 1,4-addition to a diene system extending from C₅ to C₉. Degradation studies⁹⁶ lend definite support to the view that this is actually the point of attachment of the anhydride molecule. It is of course possible that in a reaction requiring a moderately high temperature a rearrangement of the bonds precedes the formation of the addition product, and this explanation has

⁹¹ Dunn, Heilbron, Phipps, Samant and Spring, *J. Chem. Soc.*, 1576 (1934).

⁹² Auer and Walter, *Nachr. Ges. Wiss., Göttingen*, 101 (1931).

⁹³ Schulze, *Z. physik. Chem.*, A171, 436 (1931).

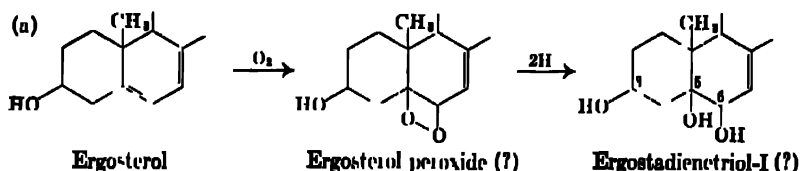
⁹⁴ Windaus and Brunko, *Ann.*, 460, 225 (1926).

⁹⁵ Windaus and Löffelringhaus, *Ber.*, 64, 570 (1931).

⁹⁶ Windaus and Inhoffen, *Ann.*, 510, 260 (1934); Inhoffen, *ibid.*, 508, 61 (1933); *Ber.*, 68, 973 (1935).

been advanced in reconciling the Heilbron formula with the observed addition. Some rearrangement probably does occur, for products are formed other than ergosterol-maleic anhydride, but it is of more significance that ergosterol can be regenerated almost quantitatively in a pure state by heating the addition product at 250° in vacuum (Inhoffen,⁹⁶ 1933). The return of the double bonds to their original positions without the formation of stereoisomers would seem a remote possibility. Since the presence of a conjugated system is indicated both by entirely valid chemical evidence and by the characteristic absorption spectrum of the sterol, the Windaus formula can be accepted as completely established.

Ergosterol Peroxide and Dehydroergosterol. When an aerated alcoholic solution of ergosterol is exposed to visible light in the presence of a sensitizing dye such as eosin, the sterol is converted into a nicely crystalline peroxide.⁹⁴ The substance is sensitive to acids, but not to alkalis, and it can be converted by reduction with zinc and alcoholic alkali to "ergostadienetriol-I,"⁹⁷ which is isomeric with the triol-II obtained from ergosterol oxide (page 172). In further work at the Göttingen laboratory, Achtermann⁹⁸ found that the triol-I rearranges to the triol-II when warmed with maleic anhydride, and he regarded this as evidence that the isomerism is of a stereochemical nature and that both substances have the 3,5,6-arrangement of the hydroxyl groups. This view has been incorporated in the formulations given by Heilbron⁹¹ and by Müller⁹⁹ (Göttingen), in which ergosterol is represented as forming a 1,2-peroxide. In terms of the Windaus formula for the sterol the changes are repre-



sented (Müller) as in (a). The isomerism of the triols I and II is assumed to be of the cis-trans decalin type.

This formulation is open to certain objections. Although represented as having two secondary hydroxyl groups, ergostadienetriol-I forms only a monoacetyl derivative (Achtermann⁹⁸), whereas the triol-II forms a diacetyl compound. Furthermore ergosterol peroxide differs from known 1,2-peroxides¹ in being stable toward alkaline reagents, and it has more the character of a transannular compound formed by the 1,4-addition

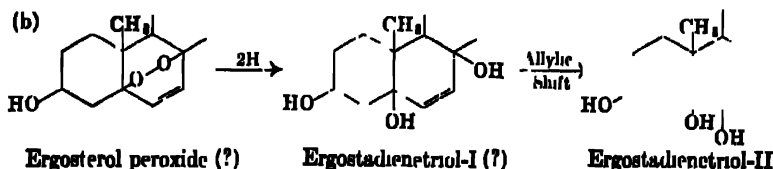
⁹⁶ Windaus and Linvert, *Ann.*, **465**, 148 (1924); Windaus, W. Bergmann and Lüttringhaus, *ibid.*, **472**, 195 (1929).

⁹⁷ Achtermann, *Z. physiol. Chem.*, **217**, 281 (1933).

⁹⁸ M. Müller, *ibid.*, **231**, 75 (1935).

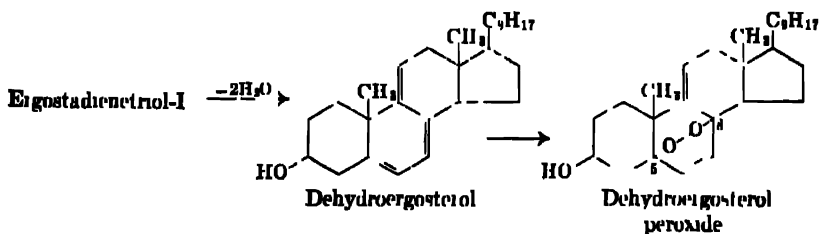
¹ Kohler, *Am. Chem. J.*, **36**, 177 (1906).

of oxygen. Such a formulation (b) appears admissible, and it accords well with the observation that the triol-I has but one group capable of being acetylated. According to this view the triols I and II are structural



isomers and the conversion of the labile into the stable isomer may occur by an allylic rearrangement.

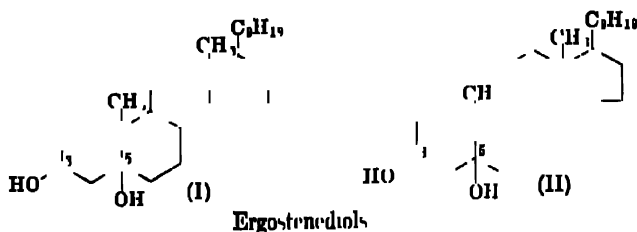
It is also consistent with formulation (b) that ergostadienetriol-I on distillation loses two molecules of water, corresponding to the two tertiary hydroxyl groups, and gives a tetra-unsaturated substance, dehydroergosterol. Ergostadienetriol-II is more stable and can be distilled unchanged, but it can be converted into dehydroergosterol by the pyrolysis of the monobenzoate or the diacetate (Achtermann⁹⁶). The highly unsaturated alcohol has been obtained also by the action of mercuric acetate on ergosterol (Windaus and Linsert⁹⁷). Although the various reactions are not easily visualized, it appears probable that the driving force in the formation of dehydroergosterol comes from the tendency to form a system of extended conjugation. The presence of a conjugated system is clearly indicated by a characteristic absorption spectrum, and by the ability of the substance to combine with maleic anhydride and to form a peroxide. According to the formulas suggested by Muller⁹⁸ the compound differs



from ergosterol only in the presence of a conjugated double bond in ring C, and the peroxide is formed by transannular addition. One indication of the presence of a conjugated system extending from C_5 to C_8 is furnished by Honigmann's² observation that the maleic anhydride addition products from ergosterol and from dehydroergosterol are converted into identical substances on the absorption of two and three moles of hydrogen, respectively.

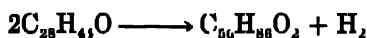
² Honigmann, *Ann.*, 508, 89 (1934).

The argument is subject to the uncertainty that a migration of the bonds is not precluded in the addition of maleic anhydride, but Müller found more secure evidence on this point on investigating the isomeric ergostenediols which had been obtained¹⁷ by the catalytic hydrogenation of the peroxides of ergosterol and of dehydroergosterol. Müller regards these diols as differing only in the location of the lone double bond, as in formulas I and II, the evidence being that they can be converted into



identical derivatives in which the substituents at C₁ and C₂ are still intact. That the tertiary hydroxyl group of the diol from ergosterol peroxide, and consequently that of the isomeric diol, is located at C₆ is shown by the relationship between the peroxide and oxide of ergosterol, and therefore one end of the peroxide chain of dehydroergosterol is definitely located at C₆. Müller believes that the other end of the chain is linked at C₈ because the peroxide, unlike dehydroergosterol itself, does not show the selective absorption characteristic of a conjugated compound. His formula for the peroxide indicates that the original conjugation has been destroyed by the 1,4-addition of molecular oxygen.

Neoergosterol. While ergosterol is converted into a peroxide when an alcoholic solution containing eosin is exposed to visible light in the presence of oxygen, an entirely different reaction occurs in the absence of oxygen.³ The sterol suffers dehydrogenation and yields a sparingly soluble substance of unknown structure called ergopinacol, the hydrogen decolorizing the dye:



On submitting ergopinacol to distillation at reduced pressure, methane is liberated and neoergosterol can be isolated from the distillate in 30% yield.

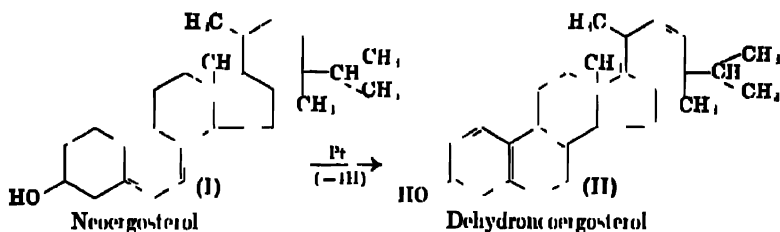
The structure of neoergosterol (I) is established by the following observations. The substance gives no sterol color reactions and contains but one reactive double bond.⁴ The isolation of methylisopropylacetaldehyde as a product of ozonization⁵ proves that the reactive linkage is situ-

¹⁷ Windaus and Borgsma, *Ann.*, **460**, 235 (1928).

⁴ Bonstedt, *Z. physiol. Chem.*, **185**, 165 (1929).

⁵ Lohoffen, *Ann.*, **497**, 130 (1932).

ated at C_{22} - C_{23} in the side chain, and since analyses point to the presence of three additional (inert) double bonds, it is inferred that one of the rings is benzenoid. That the substance yields mellophanic acid on oxidation with nitric acid (Inhoffen³) is in accordance with this conception of



the structure. Mellophanic acid could arise only as a fragment from ring B or ring C, and Honigsmann⁴ was able to distinguish between the two possibilities by a dehydrogenation experiment. When heated at 300° with finely divided platinum, cyclohexane derivatives ordinarily can be aromatized unless the process is obstructed by the presence of an alkyl group at a bridge head⁵ (as, for example, at C_{11}). Neurogosterol was converted smoothly without loss of carbon atoms to a substance (II) having the properties of a naphthol. If ring C had been originally aromatic, such a dehydrogenation would have been impossible.

Vitamin D and the Irradiation Products of Ergosterol. Rickets, a disease of infancy or early childhood characterized by faulty ossification due to defective deposit of calcium phosphate at the growing ends of the bones, has long been known to respond favorably to treatment either with sunlight, or with cod liver oil and other fish oils added to the diet. Studies of experimental rickets in rats led to the recognition that the disease is due primarily to a nutritional deficiency, and the remedial quality of cod liver oil was traced to the presence of a fat-soluble principle now known as vitamin D. The antirachitic properties were at first incorrectly attributed to vitamin A, which also concentrates in the fat-soluble, unsaponifiable fraction of cod liver oil. The special activity of the fraction is retained, however, after the removal of vitamin A, most conveniently by means of its addition product with maleic anhydride.⁶ Vitamin D is more closely related in properties to the sterols, although it is not precipitated by digitonin. The alcoholic nature of the substance has been demonstrated by Ender,⁷ who found that vitamin D reacts readily with phthalic anhydride, presumably to form an acid ester. These obser-

⁴ Honigsmann, *Ann.*, **511**, 202 (1934)

⁵ Zelinsky, *Ber.*, **44**, 3121 (1911); **45**, 3878 (1912), **56**, 1716 (1923)

⁶ Dalmer, v. Wörner and Mull, *Z. physiol. Chem.*, **224**, 86 (1934)

⁷ Ender, *Z. Vitaminforsch.*, **2**, 241 (1933)

vations have been of value in preparing purified D-concentrates from tunny liver oil (Ender) and cod liver oil,¹⁰ but although it has been possible to achieve an enrichment of about 20,000-fold the natural vitamin D has not yet been isolated in a crystalline condition. The most active preparations are composed of carbon, hydrogen, and oxygen and they display little or no optical activity.

The beneficial effects of exposure to sunlight or ultraviolet light in the treatment of rickets formed the basis of a second line of attack. In 1924 it was discovered independently by Hess and by Steenbock that, in place of irradiating the patient, the vitamin deficiency of the diet can be remedied by irradiation of the foodstuffs. It was soon found that in this manner inert oils can be endowed with antirachitic activity similar to that naturally possessed by the fish oils. As in the case of cod liver oil the activated material was found in the unsaponifiable sterol fraction of the oils. It was inferred that an inactive sterol or sterol-like substance present both in the foodstuffs and under the skin is transformed into vitamin D under the influence of ultraviolet light, and a search was made for this parent substance or provitamin. Because of the widespread occurrence of cholesterol the irradiation of this substance was investigated and the early results indicated that it is the provitamin. It became apparent, however, that, although samples of cholesterol often can be activated by irradiation even after rather extensive purification, the results are irregular, and in 1926 it was proven simultaneously in three laboratories¹¹ that the provitamin is not cholesterol but a persistent impurity. By extensive purification through the dibromide, by treatment with oxidizing agents, or by adsorption of the impurities on activated charcoal it is possible to obtain cholesterol which is not activatable. The methods found suitable for the destruction of the provitamin suggested that it is more reactive and more easily oxidized than cholesterol, and studies of the ultraviolet absorption spectra of activatable and non-activatable cholesterol indicated that the important impurity shows selective absorption characteristic of a highly unsaturated substance. Attention was then directed to ergosterol, since this was recognized as the most highly unsaturated and the most easily oxidized of the sterols, and in 1927 it was announced¹² that ergosterol is the precursor of the antirachitic vitamin. The identification was based upon chemical studies, observations of absorption spectra, and biological assays. The antirachitic potency of irradiated ergosterol is far greater than that of irradi-

¹⁰ Rygh, *Nature*, 136, 396 (1935).

¹¹ Pohl, *Nachr. Ges. Wiss., Göttingen*, 142 (1926); Heilbron, E. D. Kamm and R. A. Morton, *Chemistry and Industry*, 45, 932 (1926); Rosenheim and Webster, *ibid.*, 46, 932 (1926).

¹² Pohl, *Nachr. Ges. Wiss., Göttingen*, 185 (1927); Windaus and Hess, *ibid.*, 175 (1927); Rosenheim and Webster, *Biochem. J.*, 21, 389 (1927).

ated (crude) cholesterol, and it is considered probable that cholesterol invariably is accompanied by small amounts of the light-sensitive sterol or a similarly constituted substance.

The isolation of the antirachitic irradiation product of ergosterol in a pure form presented unusual difficulties, and it was soon recognized that under the influence of ultraviolet light the sterol is transformed not into a single substance but into a mixture of several isomers.¹³ The first crystalline substances isolated,¹⁴ suprasterol I and II, proved to be physiologically inactive products of over-irradiation. The isolation of crystalline preparations of antirachitic potency was announced in 1930-31 in both England¹⁵ and Germany,¹⁶ and the products were called calciferol and vitamin D₁, respectively. In each case the crystallizate was later found to be unhomogeneous and the isolation of the completely pure, active isomer, was reported briefly by Windaus and Linsert¹⁷ and fully described in 1932 by both the German¹⁸ and the English¹⁹ investigators. The latter workers retained the name calciferol for the new preparation, while at Göttingen this was called vitamin D₂. The earlier preparation, vitamin D₁, was found to be a molecular compound of vitamin D₂ and the isomer lumisterol.²⁰ A fifth isomer, a very sensitive substance which is known only in the form of derivatives, was called tachysterol²¹ (*Gr. tachys*, swift) because of the rapidity with which it forms an addition compound with citraconic anhydride.

Of the five well-characterized, isomeric irradiation products only vitamin D₂ or calciferol possesses antirachitic activity. When properly standardized the substance has valuable medicinal qualities, and the physiological effect is so similar to that of the preparations from cod liver oil that it was at first assumed that the irradiation product, vitamin D₂, is the active principle of the fish oils. Careful investigation, however, has revealed distinct differences in the biological actions of vitamin D₂ and natural vitamin D. A striking disparity has been noted on comparing the substance from ergosterol with highly purified D-concentrates from tunny liver oil (Ender⁹) and cod liver oil (Rygh¹⁰). While vitamin D₂ is strongly dextrorotatory, $[\alpha]_D + 103^\circ$, and the spectrum displays a maximum of absorption at 265 m μ , the fish oil preparations are optically inactive, or nearly so, and show no absorption maximum in the region

¹³ Windaus, *Nachr. Ges. Wiss., Göttingen*, 36 (1930)

¹⁴ Windaus, Gaebe, Koser and Stein, *Ann.*, 483, 17 (1930)

¹⁵ Ankew, Bourdillon, Bruce, Jenkins and Webster, *Proc. Roy. Soc. (London)*, B107, 76 (1930); Angus Ankew, Bourdillon, Bruce, Callow, Fiehmman, Philpot and Webster, *ibid.*, B108, 340 (1931)

¹⁶ Windaus, Lüttringhaus and Deppe, *Ann.*, 489, 252 (1931)

¹⁷ Windaus and Linsert, *ibid.*, 489, 269 [note added to the proof] (1931)

¹⁸ Windaus, Linsert, Lüttringhaus and Weidlich, *ibid.*, 492, 226 (1932).

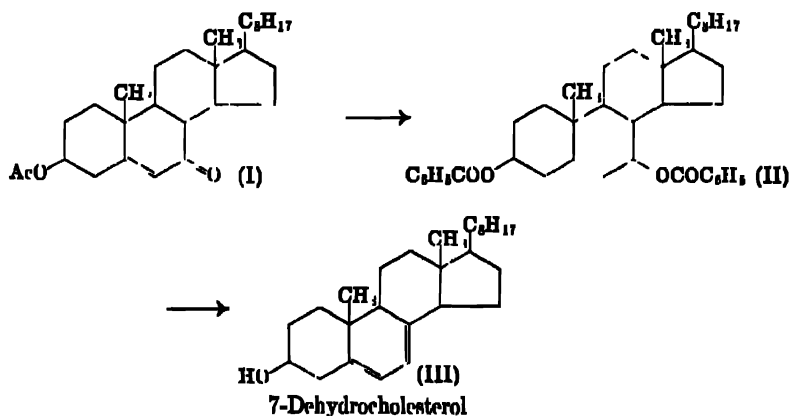
¹⁹ Ankew, Bourdillon, Bruce, Callow, Philpot and Webster, *Proc. Roy. Soc. (London)*, B109, 488 (1932)

²⁰ Windaus, Dithmar and Fernholz, *Ann.*, 493, 350 (1932)

²¹ Windaus, v. Werder and Lüttringhaus, *ibid.*, 499, 188 (1932).

260-270 m μ . On the other hand the D-concentrates from fats of various species of animals appear to be identical.²² While the possibility that the two antirachitic substances are the same perhaps must remain open until the isolation of the natural vitamin has been accomplished, the evidence at present definitely points in the opposite direction.

If the irradiation product of ergosterol is indeed not the substance originally sought, it is a good substitute for the natural vitamin. Windaus recently has made the important discovery that other potent antirachitic agents can be produced artificially. The supposition that the conjugated nuclear double bonds of ergosterol are responsible for both the characteristic ultraviolet absorption and the ability to become activated on irradiation, was substantiated by a study of 22-dihydroergosterol,²³ a compound readily prepared by blocking the conjugated system with maleic anhydride, hydrogenating the C₂₂-C₂₃ double linkage, and eliminating the maleic anhydride. It was found that the dihydro compound has nearly the same rotation and absorption spectrum as ergosterol and can be activated by ultraviolet light. The antirachitic action of the product was only about one-thirtieth that of vitamin D₂, but the observation showed clearly that ergosterol itself is not the only substance capable of functioning as a provitamin. It seemed possible that the introduction of a second double bond into cholesterol might give a substance capable of being activated, and the preparation of such a compound was accomplished by Windaus, Lettré and Schenck²⁴ in the following manner. 7-Ketocholesteryl acetate (I), prepared by the oxidation of cholesteryl acetate with chromic acid,²⁵ was reduced with aluminum isopropylate and the diol was isolated as the dibenzoate, II. When



²² Rygh, *Nature*, 136, 552 (1935).

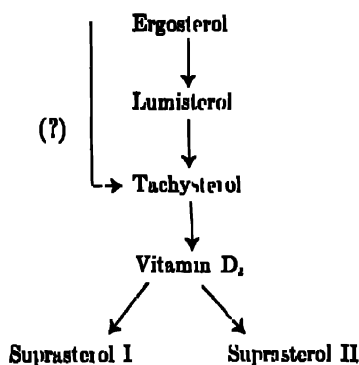
²³ Windaus and Langr, *Ann.*, 508, 105 (1933).

²⁴ Windaus, Lettré and Schenck, *ibid.*, 520, 99 (1935).

²⁵ Mauthner and Suda, *Monatsh.*, 17, 593 (1886).

heated to 200°, II lost one molecule of benzoic acid and gave the benzoate of 7-dehydrocholesterol (III). The compound has the absorption spectrum characteristic of ergosterol and the product of irradiation was found to have a remarkably high antirachitic activity. The limiting antirachitic dose for the crude material is about 0.15 γ (1 γ = 0.001 mg.), in comparison with the dosage 0.075 γ for ergosterol. The ability to acquire antirachitic properties on irradiation is not specific to a particular sterol skeleton, but appears to be dependent upon the presence of the characteristic conjugated system in ring B. This important observation leaves little support for the early view that vitamin D is necessarily identical with vitamin D₂ because of a similar physiological action.

Studies of the succession of changes occurring on the irradiation of ergosterol have shown^{21,26} that the order of formation is as follows:



The irradiation of any substance in the list leads to the formation of a mixture of all of the substances below it, and there is no indication that any of the changes are reversible. The products of over-irradiation, suprasterol I and suprasterol II, are not altered by further exposure to ultraviolet light and they are not interconvertible. There is evidence, both spectrographic and toxicologic, that another product, called "toxis-terol" or "substance 248," is produced by over-irradiation,²⁷ but the substance has not been isolated. In addition to this succession of products of irradiation, two additional isomers have been obtained by thermal treatment of vitamin D₂: pyrocalciferol¹⁹ and isopyrovitamin²⁸. While this remarkable series of well-characterized isomeric transformation products is perhaps without a really comparable parallel, it may be noted that there are some points of similarity in this respect between ergosterol and abietic acid. In each substance there is a cyclohexane ring contain-

²¹ Setsu, *Z. physiol. Chem.*, **215**, 143 (1933).

²⁷ R. A. Morton, Heilbron and E. D. Kamm, *J. Chem. Soc.*, 2000 (1927); Billis, Honeywell and Cox, *J. Biol. Chem.*, **80**, 657 (1923); Laquer and Lannert, *Klin. Wochenschr.*, **12**, 753 (1933).

²⁸ Rumer, *Z. physiol. Chem.*, **214**, 211 (1933).

ing two double linkages, probably in positions of conjugation, and it will be recalled that abietic acid is only one of a series of rather labile isomers. These isomerizations have been little studied, however, and the changes have been induced only by heat or with the use of catalysts.

In view of the difficulty of establishing the complete structure of ergosterol, it is hardly surprising that the problem of accounting for the products of irradiation is not yet completely solved. Considerable progress has been made in the brief period since the discovery of the compounds, and in anticipation of a rapid development of the field in the near future it will be sufficient to summarize briefly the secure advances already made. The five products of irradiation and the two pyro-compounds listed in the accompanying table are all isomers of ergosterol. Unlike the parent compound, the irradiation products are not precipitated by digitonin, and isopyrovitamin is precipitated only incompletely by the reagent. The double linkage at $C_{22}-C_{23}$ is not involved in the transformations, for the first five compounds all yield methylisopropylacetaldehyde on ozonization²⁹. It can be inferred that the absorption of ultraviolet light is attended with changes in the highly unsaturated ring B of ergos-

ISOMERIC TRANSFORMATION PRODUCTS OF ERGOSTEROL

	M p	Number of double bonds	Number of rings	Crystalline dehydro-oxidation products	Oxidation to methylbutyric or itaconic acid	Evidence of conjugation
Ergosterol	163°	3	4	$C_{18}H_{16}$	Positive	Positive
Lumisterol	118°	3 ^{30, 31}	4	$C_{18}H_{16}$ ³⁰	Positive ³⁴	
Tachysterol		4 ³²	3		Negative ³⁷	Positive ²¹
Vitamin D ₂ (Calciferol)	116°	4 ^{33, 34, 35}	3	None ^{32, 37}	Negative ³⁵	Positive ³⁷
Suprasterol I	104°	3 ^{35, 36}	4	None ³⁷		Negative ³⁷
Suprasterol II	110°	(7) ^{35, 36}	3 or 4	None ³⁷		Negative ³⁷
Isopyrovitamin		3 ^{28, 36}	4	$C_{18}H_{16}$ ³⁷	Positive ³⁷	Positive ³⁷
Pyrocalciferol	95°	3 ^{29 (2?) 30}	4	$C_{18}H_{16}$ ³⁷	Positive ³⁷	Positive ³⁷

²⁹ Guiteras, *Ann.*, 494, 116 (1932)

³⁰ K. Dunroth, *Ber.*, 68, 539 (1935)

³¹ Heilbron, Spring and Stewart, *J. Chem. Soc.*, 1221 (1935)

³² Letré, *Ann.*, 511, 290 (1934)

³³ Kuhn and E. F. Möller, *Z. anorg. Chem.*, 47, 145 (1934)

³⁴ Heilbron, Samant and Spring, *Nature*, 135, 1072 (1935)

³⁵ Windaus, Gaede, Koser and Stann, *Ann.*, 483, 17 (1930)

³⁶ Kuhn and Möller, see M. Möller, *Ref.* 37.

³⁷ M. Möller, *Z. physiol. Chem.*, 233, 228 (1935)

³⁸ Juhnken, *Ann.*, 494, 122 (1933)

³⁹ Windaus and W. Thiele, *ibid.*, 521, 160 (1935)

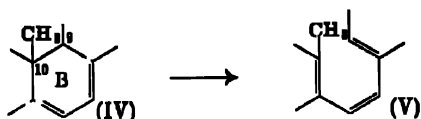
terol. In consequence it is important to determine the degree of unsaturation in the different compounds, and this has been accomplished where possible either by hydrogenation experiments, particularly by the quantitative micromethod of Kuhn, or by perbenzoic acid titrations. Lumisterol, like the parent compound, contains three double bonds and it may be inferred that the change in this case involves an epimerization or the migration of a double linkage. The presence in both tachysterol and vitamin D₂ of an additional double bond has been definitely established, and the surprising conclusion is reached that one of the four original rings has opened in the course of the photo-isomerization. Over-irradiation, at least in case of the production of suprasterol I, appears to result in the closing of a ring, for only three double linkages are indicated for the compound. The same is true for the two pyro-compounds.

Another method of determining the number of rings is by dehydrogenation experiments. Ergosterol, like cholesterol and cholic acid, is converted by treatment with selenium into the Diels hydrocarbon (C₂₈H₄₀), methyleyclopentenophenanthrene. In accordance with the conclusion reached above, lumisterol yields the same hydrocarbon and therefore contains the original ring system. In confirmation of the evidence that the four-ring system is no longer present in vitamin D₂, this substance has yielded no crystalline products of dehydrogenation in repeated experiments by different investigators. Although suprasterol I contains a four-ring system, this evidently is different from that of ergosterol. In contrast to this product of the further irradiation of vitamin D₂, the original ring system apparently has been reformed in the case of the two pyro-compounds. Although the cyclopentenophenanthrene system is indicated for lumisterol and for the two pyro-compounds there probably are stereochemical differences, for these substances yield different perhydro derivatives, all of which differ from perhydroergosterol.

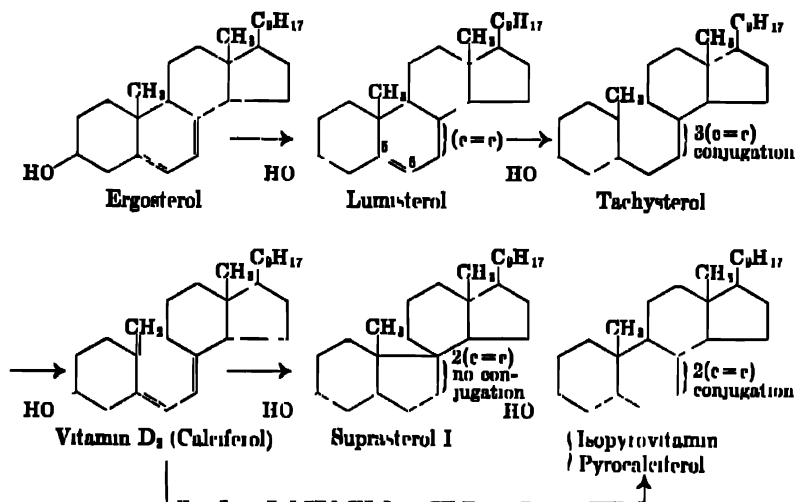
Although the oxidation of ergosterol with nitric acid must involve a peculiar rearrangement (page 172), the reaction appears to be a characteristic one and the formation of a benzenoid acid probably is indicative of a concentration of the unsaturated linkages into a single ring. As applied to the isomers of ergosterol the results parallel exactly those of the dehydrogenation experiments. In those cases in which the original ring system is retained it is probable that the nuclear double bonds are associated with ring B.

In analyzing the problem of the structures both Lettré²² and Müller²⁷ assumed that it is the unsaturated ring B which has been opened in the change lumisterol → tachysterol, a point which is supported by the failure of tachysterol to yield methylbenzene tetracarboxylic acid on oxi-

dation with nitric acid. Lettré suggested that it is the 9,10-bond which is severed, for example as in the formulation:



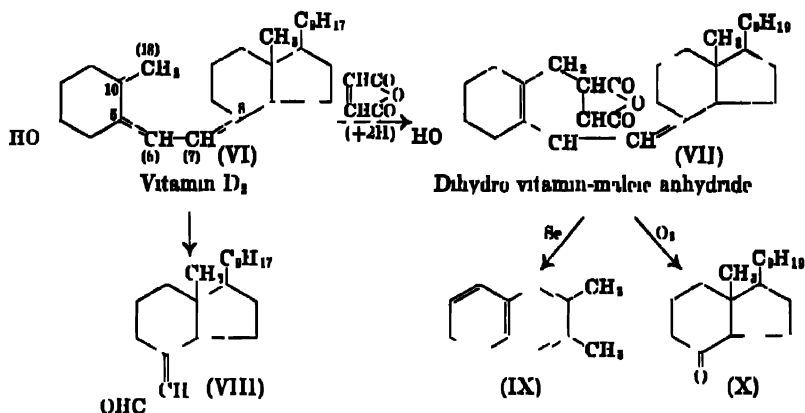
The positions of the double linkages are not all known, however, and in summarizing by means of partial formulas the information at present available concerning the structures the number of unlocated nuclear double bonds is in each case indicated in brackets. Heilbron^{31,40} found



that lumisterol gives a triol which forms only a diacetyl derivative, and by analogy with ergostadienetriol-3,5,6 it is probable that an ethylenic linkage occupies the 5,6-position as with ergosterol. The position of the second nuclear center of unsaturation is unknown. The only information available regarding tachysterol is that the double bonds must be arranged in a particularly reactive system of conjugation. Tachysterol and vitamin D₂ yield identical dihydro derivatives (Muller³⁷) and consequently possess the same carbon skeleton.

Vitamin D₂ is of course the most interesting member of the series and recent investigations have established the structure of the antirachitic substance with almost complete certainty. That the 9,10-bond is ruptured in the photochemical rearrangement of ergosterol, as in the formulas first suggested by Lettré,³² was established by Heilbron and co-

workers.^{34, 40} By the careful oxidation of calciferol (vitamin D₂), VI, the English investigators obtained an unsaturated aldehyde C₂₁H₃₄O (VIII) which, because of the number of carbon atoms and the absence of a hydroxyl group, can only arise from the cleavage of a double bond at the original 5,6-position of a structure such as that of formula VI. One of



the two unlocated ethylenic linkages must be associated with the bicyclic part of the molecule, the other with the cyclohexane ring, and Heilbron suggested provisionally the positions C₈-C₉ and C₁-C₁₀. Windaus and Thiele⁴⁰ were able to carry the evidence still further in a study of two isomeric (stereoisomeric?) addition products of vitamin D₂ with maleic anhydride. Both substances gave dihydro derivatives by the saturation of the double bond in the side chain. The German workers represented the addition as occurring to the diene system extending from C₆ to C₁₄, as shown in the formula (VII) for the dihydro compounds. This structure is firmly grounded by the following degradations. On ozonization of both of the dihydro addition products there was obtained a saturated ketone which, from the composition (C₁₆H₂₄O, bicyclic), must have the structure X. The observation establishes the presence of a double bond in the 7,8-position. The nature of the other half of the molecule was revealed by the isolation of 2,3-dimethylnaphthalene (IX) as a product of the selenium "dehydrogenation" of the dihydro addition products. The reaction appeared unusual, for although hydroaromatic compounds had been observed to undergo reduction and hydrogenation in the presence of selenium (page 159), no example was known of the conversion of carboxyl or anhydride groups into methyl groups, as demanded by the above formulation. In model experiments, however, Thiele and Trautmann⁴¹ found that such a reaction can be realized with simpler hydroaromatic

⁴¹ W. Thiele and Trautmann, *Ber.*, **66**, 2215 (1933).

anhydrides, or with a mixture of an aromatic anhydride and a hydrogen donor. *p*-Cyclohexylphenol is a convenient substance to use as a source of hydrogen atoms because the *p*-hydroxydiphenyl formed on dehydrogenation is easily separated from the reaction product. On heating naphthalene-2,3-dicarboxylic acid anhydride with selenium and *p*-cyclohexylphenol there was obtained 2,3-dimethylnaphthalene, an observation which clearly supports the interpretation of the above reaction.

The identification of degradation products characterizing both parts of the molecule establishes rigidly the structure of the maleic anhydride addition product. Provided that there is no rearrangement in the reaction of vitamin D₂ (as the acetyl derivative) with maleic anhydride at the temperature of the steam bath, these observations also completely establish the structure of the vitamin itself. From the present indications a rearrangement appears unlikely.

From the fact that lumisterol is not precipitated by digitonin it is probable that an epimerization occurs in the first step of the photoisomerization, and all of the succeeding compounds of the series are provisionally regarded as *epi*-compounds. The highly reactive tachysterol may well have the bond structure indicated in formula V, above. The transformation of the compound into vitamin D₂ would then involve a simple migration of the conjugated system to include the methyl group. The thermal cyclization of vitamin D₂ with the reformation of the sterol ring system (pyrovitamins) is understandable in the light of the Windaus formula. It is interesting that a formula very similar to that of vitamin D₂ has been suggested for *l*-pimaric acid, a primary constituent of oleoresins which is easily cyclized by heat (page 68). Suprasterol I has a ring system different from that of the sterols and Müller²⁷ suggested that in this case a spirocyclopentane ring is produced as the result of irradiating the vitamin. This change is likewise reconcilable with the newly established formula.

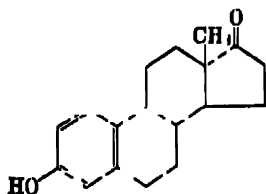
Clearly the complicated picture has assumed definite outlines and, although further investigation will be required to supply completing details, the problem of the irradiation products of ergosterol can be regarded as solved in the most essential details. The vitamin D problem is still open, however, for the natural vitamin has not been isolated and it very probably is different from vitamin D₂. The demonstration that the cholesterol molecule can be modified in such a way as to become capable of activation suggests that the animal organism may produce its provitamin from this sterol.

Chapter V

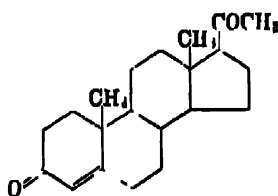
Sex Hormones

While the abundantly occurring sterols and bile acids have been the subject of chemical investigation since the very earliest days of the science, the chemistry of the sex hormones is a strictly modern problem. Certain of these hormones occur in the organism in extremely small amounts and can be separated from other biological material only with the greatest difficulty, and it was not until 1929 that work on the interesting and important problem of structure was made possible by the isolation of one member of the series in a pure, crystalline condition. The history of the problem since 1929 has been remarkable in many ways, not the least of which being that in the short space of six years sex hormones of three important types were isolated, their complicated molecular structures were completely elucidated, and methods were developed for making the pure materials available for clinical use and for biological experimentation. Considering in addition the striking biogenetic relationships which the work has disclosed, it may be said that this is one of the most spectacular achievements of organic chemistry.

Preceding the chemical researches, and furnishing the necessary foundation for this phase of the work, there was a considerably longer period of biological experimentation leading to the recognition of the existence of the hormones and to the definition of their specific functions. By purely biological methods it was established that all sexual processes of the organism proceed under the influence of certain chemical substances recognizable by specific biological tests and known as the sex hormones. The chemical work has established the compositions and the structural formulas of the principal members of the group, namely, the follicular

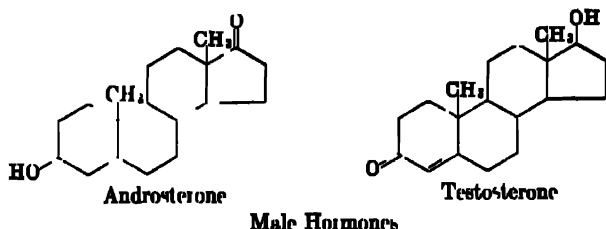


Oestrone



Progesterone

Female Hormones



hormones oestrone and oestradiol (dihydro-oestrone), progesterone, the hormone of the corpus luteum, and the male hormones androsterone and testosterone. Each hormone exists in various polymorphic forms, and each is accompanied by certain related substances, of which some are inactive and some have the same kind of physiological activity as the principal hormone of the group. Androsterone, testosterone, and all related male hormones have qualitatively similar physiological properties, but the actions of oestrone and progesterone in the female organism are entirely different. In discussing the physiological functions it is convenient to refer to the three principal groups of hormones rather than to specific members which have been isolated in a pure, crystalline condition. The term "oestrin" is applied to the group of which the pure substances oestrone and oestradiol are the typical representatives, and "progestin" designates progesterone and its possible companions of similar physiological properties present in the corpus luteum.

The sex hormones appear to be formed in the testes or in the ovaries under the stimulation of hormonal secretions (gonadotropic hormones) from the anterior lobe of the pituitary. They control the growth and the physiological functioning of the reproductive organs and, according to their nature, the hormones secreted by the genital glands promote the development of either the male or female secondary sex characteristics. The male hormones control the development of the genital tract and the accessory male organs, and they influence the longevity and the motility of the sperm. The normal growth of secondary marks of the male sex, such as the comb and wattles of the cock, takes place under the stimulation of the male hormones. Corresponding with the greater complexity of the female organism, at least two kinds of hormones appear to be required to control the various processes in the uterine cycle and in pregnancy. One type, oestrin, is produced in the ovary, possibly in the ripening follicles, and it passes from the ovary to the uterus and the vagina and produces the characteristic changes of oestrus (sexual heat). Since it usually is associated with the follicular phase, oestrin is often referred to as a follicular hormone, but it is more specifically defined as an oestrus-producing hormone. The second female sex hormone, pro-

gestin, is secreted by the corpus luteum (yellow body), an organ formed from the cells lining the follicle after rupture and expulsion of the ovum. The hormones oestrin and progesterin, acting in conjunction, control the uterine cycle. In humans this consists in the periodic preparation of the uterus for pregnancy, and it occurs in two phases. The first phase, occurring under the hormonal influence of oestrin, consists in the growth or proliferation (cell-division) of a functional bed, or mucosa, in the uterus. The corpus luteum hormone then prepares this bed for the implantation of the fertilized ovum, in the second, secretory phase. In case there is no fertilization leading to pregnancy, the mucosal bed largely degenerates and returns to the unproliferated, or resting, condition.

The Gonad-Stimulating Hormones. However important in the direct regulation of the sex processes, the hormones of the gonads (testes and ovaries) are not in complete, primary control of these processes for they owe their origin to the stimulating action of still another kind of sex hormone. This other hormone, or group of hormones, is secreted by the anterior lobe of the pituitary gland and passes in the blood stream to the testis or ovary and there stimulates these organs to produce either male hormones or oestrin and progesterin. Because of this action on the gonads, the active substances of the pituitary are called gonadotropic hormones.

That the sexual cycle is under the direct control of the pituitary was perhaps first indicated by clinical observations of the connection between certain diseases and the disfunction of the pituitary gland. A confirmation was found in the study of the partial or total removal of the anterior lobe by operation (hypophysectomy). By partial hypophysectomy it was found possible to arrest normal sexual activity, and by complete hypophysectomy all sexual activity could be inhibited (Cushing, 1909). With the development of a technique for studying the effects of operations on small female animals (Smith, 1927), it was demonstrated clearly that the removal of the anterior lobe before puberty leads to continued infantilism, while if the operation is performed at a later period the sex cycle ceases and the ovaries become atrophic. The next advance was the demonstration that the implantation of pituitary tissue into hypophysectomized female animals restores the normal cyclic changes and results in the reappearance of the state of oestrus (Smith, 1930). New follicles are produced and luteinization (corpus luteum formation) occurs. With immature normal females the implantation of such tissue leads to precocious sexual maturity: enlargement of the ovary and uterus, appearance of oestrus (Zondek and Aschheim, 1927; Smith and Engle, 1927). Implants of the pituitary gland also bring about the regeneration of the reproductive organs in hypophysectomized male animals. In the case of castrated animals such implantation has no effect. This body of

observations indicates the nature of the relationship between the anterior lobe and the gonads, and it clearly establishes the pituitary control of the asexual processes.

That this control is purely hormonal in character finally was established by the production of similar effects with cell-free extracts of the pituitary (Evans, 1928). These extracts appear to contain as the active principle a chemical substance or group of substances which under normal conditions is carried from the brain in the blood stream and stimulates the gonads to produce the hormones of the genital glands. The active principle of the hypophysis does not appear to be sex-specific like the hormones produced under its stimulation in the testis or ovary, although according to some workers the active principle can be divided into two factors, one of which is thought to induce follicle ripening, the other to promote luteinization.

The whole problem of the chemistry of the gonad-stimulating hormones still awaits solution, for the active principles have not been isolated in a pure condition. A physiological method of determining quantitatively the activity of extracts and preparations is available through the observation of changes produced in infant female mammals. A "mouse unit" of the hormone is defined as the smallest amount of substance which will bring about follicle ripening and luteinization in the infant female mouse. A source of gonadotropic hormones other than pituitary tissue has been discovered in the urine of women in the first stage of pregnancy. The urinary hormone is called prolan, and there are some differences between this material and the hormone obtained from pituitary tissue. The biological assay of prolan in urine provides a method for the early diagnosis for pregnancy (Aschheim-Zondek reaction), for the hormone is excreted in the urine only during this condition. The original Aschheim-Zondek test has been largely replaced in this country and in England by the Friedman test (rabbit ovulation). The latter requires a single intravenous injection in a sexually mature rabbit. The prolan found in urine comes from the placenta, rather than from the anterior lobe of the pituitary, and its relationship to the active pituitary substance is not yet clear. Active preparations can be obtained from the urine of pregnancy by various methods.¹ Prolan is soluble in water, dialyzable, sensitive to heat and to hydrolytic or proteolytic enzymes. It is easily adsorbed on benzoic acid, quinone, or permutit, and it can be precipitated from an alcoholic solution by means of acetone.

Biological Investigations of the Male and Female Sex Hormones. The presence of hormones in the testis and ovary, and the nature of their

¹ Katsamen and Doney, *J Biol Chem*, 96, 739 (1932), Haurowitz, Rowe and Balint, *Z physiol Chem*, 222, 41 (1933)

specific physiological functions was established by animal experiments resembling in general purport those described above and for the most part antedating the work on the gonadotropic hormones. With the use of castrated animals, or animals not sufficiently mature to display full sexual activity, it was possible to test various biological materials for their ability to restore or to initiate such activity. Since the complete removal of the ovaries (double ovariectomy) from a female mammal abolishes the normal cyclic changes, these changes must be due to some influence from the ovaries. That the effect is hormonal in nature, that is, due to the stimulating action of some agent capable of being transported in the body fluid, was inferred from the observation that the cyclic changes are maintained on autotransplantation of the ovaries to other sites of the body. A great advance was made in 1923 by E. Allen and Doisy,² who discovered a convenient test for the follicular hormone which depends upon its ability to produce the typical oestrous reaction when injected into castrated mice or rats. A positive reaction is easily recognized, for the reproductive cycle in the normal animal is characterized by distinct changes in the cell structure of the lining of the vagina. At the height of the oestrous state this acquires a unique, cornified character easily distinguished from that typical of the resting period, or the permanent condition of the castrated animal. The microscopic examination of vaginal smears gives a reliable indication of the oestrous condition of the living animal.

Allen and Doisy prepared alcoholic (cell-free) extracts of ovaries capable of inducing typical oestrus in the test animals, and in this way proved that the active principle is a chemical substance. It was possible, moreover, to evaluate the activity of a preparation in terms of mouse or rat units. A mouse unit of the hormone is defined as the quantity which just suffices to produce the oestrous response in the castrated animal. The amount of material required depends somewhat upon various details of the procedure, and the mouse unit, as defined in the standard procedures employed in different laboratories, varies from about 0.04 γ to about 0.1 γ of pure oestrone (1 γ =0.001 mg.). The method of bio-assay gives very reliable and reproducible results³ and, since the sexual cycle for the mouse is only 4-6 days in duration, the test can be performed rapidly. Many investigators prefer to determine activity in terms of rat units. A comparison of results is rendered uncertain by the fact that the ratio of the mouse unit (m. u.) to the rat unit (r. u.) varies

² E. Allen and Doisy, *J. Am. Med. Assoc.*, 81, 819 (1923).

³ Kahut and Doisy, *Endocrinology*, 12, 700 (1928); Butenandt and v. Ziegner, *Z. physiol. Chem.*, 188 1 (1930); Gad-Andersen and Jarlov, *Acta Med. Scand.*, 84, 224 (1934).

considerably depending upon the medium and the method of injection. Most authorities place this ratio somewhere within the limits 1:4 to 1:9.

The Allen-Doisy test proved to be an indispensable guide in the search for the pure oestrus-producing hormone. The substance was found present in the follicular fluid, in the mammalian placenta, and in the blood, and fairly active extracts of the first two tissues were prepared by a number of different investigators. Organic solvents extracted a number of substances in addition to the hormone, however, and a separation from the inert material of the tissues proved to be extremely difficult. Little progress in the direction of the isolation of the hormone was made until Aschheim and Zondek ⁴ in 1927 made the important discovery that an oestrus-producing substance is excreted in considerable quantities in the urine of pregnant women. This observation was of enormous value in expediting the chemical investigations, for a simple benzene or ether extraction of the urine of pregnancy provides a solution of a large quantity of the hormone in a far purer condition than it had been possible to obtain by elaborate purification of the alcoholic tissue extracts. In the urine the substance is not contaminated with the great mass of inert materials extracted from the ovaries. With a convenient source of the hormone and a reliable test for physiological activity both available, the work of isolation entered a final, chemical phase and in 1929 a follicular hormone was obtained in a pure, crystalline condition by two different groups of investigators. This work will be described below.

The investigations of the male sex hormone ⁵ date at least to the experiments of Berthold in 1849. The castration of a cock leads to the general regression of the characteristic head furnishings of the animal, and the comb and wattles soon atrophy and almost completely disappear. Berthold found that the transplantation of testis tissue causes the comb of the capon to resume growth. The regeneration of secondary sex characteristics by the implantation or grafting of testicular tissue was clearly demonstrated in later experiments with the same test animal (Pézar, 1911), but it was not until 1927 that the effect was proved to be of a hormonal character. In that year McGee, ⁶ of the Chicago research group of Koch and Moore, prepared a cell-free, alcoholic extract of bull testes containing an active principle capable of promoting comb-growth in capons. The observation was soon confirmed and extended by a number of other investigators, (Koch and Moore, Loewe and Voss, Funk and Harrow, Steinach, Dadds, Laqueur, Frattini and Maino) but at first all

⁴ Aschheim and Zondek, *Klin. Wochschr.*, 6, 1322 (1927).

⁵ For detailed discussions and references, see C. R. Moore, "The Biology of Testis," and F. C. Koch, "Biochemistry and Assay of Testis Hormones," in F. Allen's "Sex and Internal Secretions," pp. 291-391 (1932).

⁶ McGee, Dissertation, Chicago (1927).

attempts to isolate the active principle from this source were fruitless. Prompted by the possible analogy to the follicular hormone, a number of workers then investigated the normal urine of males and found⁷ (1928) that a male hormone is excreted in the urine, although only in extremely small amounts. Following shortly after this discovery the difficult task of isolating a hormone in a pure condition was accomplished in 1931. Finally, in 1935, a male hormone was isolated from testicular extracts.

Also of recent date is the work on the corpus luteum hormone. A biological test (Corner and Allen, 1929) was found in the effect of the active principle of the corpus luteum on the uterine mucosa of the sexually mature castrated rabbit, but the absence of any source of material other than the corpus luteum itself was a great handicap in the investigations. The isolation of progesterone was achieved in 1934

THE OESTROGENIC HORMONES

Isolation and Properties of Oestrone. The isolation of this important oestrus-producing hormone in a pure, crystalline condition was accomplished independently in 1929 by Doisy and co-workers⁸ at the St. Louis University School of Medicine and by Butenandt⁹ at Gottingen. The respective results were announced at Boston in August and at Kiel in October. Early in the following year the Laqueur research group¹⁰ at Amsterdam reported the isolation of an apparently identical crystalline substance of high physiological activity. In each case the urine of pregnant women was used as the starting material. When such urine is shaken with an immiscible solvent such as ether, butanol, or benzene a considerable portion of the oestrogenic material is extracted, for the hormone is readily soluble in all organic solvents and sparingly soluble in water. A better yield is obtained if the urine is acidified and submitted to hydrolysis before the solvent extraction. Fresh urine is not required, as the hormone does not appear to deteriorate rapidly. In the first step of Doisy's original process¹¹ the urine was acidified to pH 4, allowed to stand for several days, and extracted with olive oil. The hormone was then extracted with alcohol. Butenandt made use of a technical crude oil supplied by the Schering-Kahlbaum A.-G. This was obtained by the ether extraction of acidified urine, followed by the partial removal of acidic impurities by extraction with very dilute alkali. The biological assay of the dark brown syrup indicated the presence of about 0.3 per cent of active mate-

⁷ Loewe, Voss, Lange and Wanner, *Klin. Wochschr.*, 7, 1376 (1924).

⁸ Doisy, Volar and Thayer, *Am. J. Physiol.*, 90, 320 (1929), *J. Biol. Chem.*, 86, 490 (1930), 87, 387 (1930).

⁹ Butenandt, *Naturwissenschaften*, 17, 879 (1929), Butenandt and v. Ziegner, *Z. physiol. Chem.*, 188, 1 (1930).

¹⁰ Ding-manno, de Jongh, Kober and Laqueur, *Deut. med. Wochschr.*, 56, 301 (1930).

¹¹ For later methods, see Katsman and Doisy, *Proc. Soc. Exptl. Biol. Med.*, 30, 1196 (1933).

rial. In the various methods for the further purification of these crude extracts, advantage is taken of the stability of the hormone to acids, bases, and heat, and of its weakly acidic (phenolic) character and ketonic properties. After an alkaline hydrolysis of the crude oil, Butenandt¹² distributed the material between 50 per cent alcohol and ligroin, the first solvent retaining nearly all of the hormone. A further enrichment was attained by extracting the active principle from the alcoholic phase into benzene. After removal of the solvent the material was subjected to hydrolysis with hot, alcoholic hydrochloric acid and extracted from a solution in ether with 1 *N* sodium hydroxide. The purified oil then yielded a crude, yellow crystallizate on distillation in high vacuum, and completely pure crystals were obtained on repeated sublimation and crystallization. It was later found¹³ that the hormone forms with quinoline a very sparingly soluble molecular compound which affords a convenient method of obtaining pure, colorless crystals from the crude distillate. The complex crystallizes almost completely from quinoline and it is decomposed by shaking with ether and dilute acid. The semicarbazone also has been used in isolating and purifying the hormone.¹⁴

As stated above, treatment of human pregnancy urine with acid markedly increases the yield of hormone which can be extracted with solvents, and for the highest yield hydrolysis under drastic conditions is required. This was demonstrated clearly in the systematic studies of the Laqueur group.¹⁵ It is not a question of liberating oestrone and its companion oestriol (page 200) from their simple (phenolic) salts, but of decomposing stable combinations of the substances with constituents of urine (glucuronic acid?). Cohen and Marrian,¹⁶ in a careful study of the factors influencing hydrolysis, found that in order to make an accurate quantitative assay of the oestrone and oestriol in pregnancy urine it is necessary to effect the hydrolysis under conditions which combine maximum liberation of the hormones from the ether-insoluble forms with minimum destruction of the liberated substances. They recommend that the urine be adjusted to pH 1, further acidified by the addition of 3.3 cc. of 12 *N* hydrochloric acid per 100 cc. of urine, and autoclaved at 120° for two hours.

In this work Cohen and Marrian (1934) employed a colorimetric method, based on the Kober test,¹⁷ for the separate quantitative estimation of oestrone and oestriol in ethereal extracts of human pregnancy urine.

¹² Butenandt, *Z. physiol. Chem.*, **191**, 127 (1930).

¹³ Butenandt and U. Westphal *ibid.*, **223**, 147 (1934).

¹⁴ Curtis, MacCorquodale, Thayer and Douse, *J. Biol. Chem.*, **107**, 191 (1934).

¹⁵ Borchardt, Dingemans and Laqueur, *Naturwissenschaften*, **22**, 190 (1934).

¹⁶ S. L. Cohen and Marrian, *Biochem. J.*, **28**, 1603 (1934); **29**, 1577 (1935).

¹⁷ Kober, *Biochem. Z.*, **239**, 209 (1931); *Acta Brevia Newland*, **5**, 34 (1935).

The Kober method, which depends upon the development of a red color when the hormones are heated with phenolsulfonic acid-sulfuric acid followed by the addition of water, has been simplified by Cartland, *et al.*,¹⁸ who, like Cohen and Marrian, found that the results are in good agreement with the bio-assays. David¹⁹ has described a color test specific for oestriol, and Zimmermann²⁰ has investigated the use of the Jaffe picric acid reaction for the colorimetric determination of ketonic hormones.

The properties of pure oestrone are indicated in the accompanying table. Studies²¹ of the crystalline form and of the melting point on the

PROPERTIES OF OESTRONE

Formula	M. p. ²² (corr.)	$[\alpha]_D^{25}$	Solubility in water ¹³ (18°)	Dissociation constant ¹³	Physiol activity, mouse units per g. ²⁴
$C_{18}H_{22}O_2$	259°	+ 158.5°	2 mg./liter	0.44×10^{-9}	8-10 million

Derivatives,²⁴ m. p. Acetate, 126°; Benzoate, 217.5°; Oxime, 233°.
Methyl ether, 167°; Semicarbazone 259°

microscopic stage indicate that oestrone can exist in no less than three polymorphic forms, melting at 254°, 256°, and 259° (corr.). This observation probably accounts for the divergence in the melting points recorded in the literature. At one time Butenandt²⁵ interpreted small differences in the physical properties and bio-assay of samples obtained from different sources as indicating the existence of isomeric forms (α and β) of the hormone, but the burden of evidence^{14, 22, 2'} does not support this view. An apparently isomeric substance (δ -follicular hormone) of much lower melting point and physiological activity was described by Schwenk and Hildebrandt.²⁶

The hormone has been known by a number of different names and until recently little accord has been reached in this matter. Doisy's designation "thelin" (Gr. *thelos*, female) has been used widely in this country, but it was applied at a time when the structure of the hormone was not known and the name unfortunately does not reflect the chemical characteristics of the substance. Butenandt soon withdrew his original sug-

¹⁸ Cartland, R. K. Meyer Miller and Rutz, *J. Biol. Chem.*, **109**, 213 (1935).

¹⁹ David, *Acta Brava Newland*, **4**, 64 (1933).

²⁰ Zimmermann, *Z. physiol. Chem.*, **239**, 237 (1935).

²¹ A. Koller and Hauerhild, *ibid.*, **224**, 150 (1934), *Mikrochemie*, **15**, 55 (1934).

²² Girud, Sandulacn, Fridenson and Rutgers, *Compt. rend.*, **194**, 90ⁿ (1932).

²³ de Jongh, Kober and Laqueur, *Biochem. Z.*, **270**, 17 (1934).

²⁴ Butenandt, in Richter-Anschütz, "Chemie der Kohlenstoffverbindungen," II, 575 (1935).

²⁵ Butenandt and Zimmer, *Z. physiol. Chem.*, **208**, 129 (1932).

²⁶ Schwenk and Hildebrandt, *Naturwissenschaften*, **20**, 658 (1932), *Biochem. Z.*, **259**, 240 (1933).

gestion of "progynon" and employed the somewhat noncommittal, but perhaps too specific, term "follicular hormone," and later " α -follicular hormone." The specification of the " α -form" has lost its original significance. Marrian used "oestrin," a name applied to the crude extract by Parkes in 1926, and "ketoxyhydroxyoestrin." Laqueur named the substance "menformon," while Girard called it "folliculin." In an effort to promote general agreement by abandoning names bearing an implication of priority claims, a group of English investigators²⁷ in 1933 proposed the name "oestrone," which indicates the ketonic character of the hormone and its most important physiological action. The proposal included a general system of nomenclature according to which the alcohol resulting from the reduction of the hormone is called "oestradiol," while a companion substance having three hydroxyl groups is known as "oestriol." The English system was informally accepted by the majority of the investigators in the field at the League of Nations Conference held in London in the summer of 1935,²⁸ and it is adopted in this book. The term "oestrin" is at present rather generally used to indicate the entire group of oestrogenic substances found in urinary or tissue extracts.

Although the quantitative determination of oestrogenic activity by the Allen-Doisy method gives remarkably reproducible results, there are some variations arising from differences in the technique followed in different laboratories. In order to provide a means of comparison, an international standard preparation consisting of 20.9 g. of pure oestrone contributed by various countries was established in 1932 at the National Institute for Medical Research, London, under the auspices of the Health Organization of the League of Nations.²⁹ The international unit is the quantum of activity of 0.1 γ (1×10^{-7} g.) of the standard preparation. In making the assay a solution of the hormone in oil (sesame oil) is administered by subcutaneous injection. The dose required by mouth is about 40 times the subcutaneous dose.²⁵

Other Sources of Oestrone. The average oestrogenic activity of the urine of pregnant women is about 10,000 mouse units per liter. Calculated as pure oestrone this would correspond only to about 1 mg. of the hormone per liter, and the yield of pure material which can be extracted by the ordinary laboratory technique is only a fraction of that present. In 1930 Zondek³⁰ discovered a new and better source of the hormone in the urine of pregnant mares, the average activity of which is 100,000 m. u. per liter, or ten times that of human urine. It is estimated³¹ that a single

²⁷ Adam, Danielli, Dodds, King, Parkes and Rosenham, *Nature*, 132, 205 (1933).

²⁸ Private communication from Dr. W. M. Allen.

²⁹ *J. Am. Med. Assoc.*, 101, 877 (1933).

³⁰ Zondek, *Klin. Wochenschr.*, 9, 2295 (1930).

³¹ *Idem*, *Naturwissenschaften*, 21, 83 (1933).

mare eliminates during pregnancy as much as 30 g. of oestrogenic material. Only from about 1500 patients of maternity clinics could an equal amount of material be obtained. This abundant and rich source has greatly expedited the work of isolation. The technical production of the follicular hormone has been undertaken with success by several industrial concerns and the valuable pharmaceutical preparation is now available in quantity at a comparatively reasonable price. In the urine of the mare the hormone is present in an ether-insoluble form to an even greater extent than in the case of human urine, and only 10-25% of material is directly extractable.³² Prior to the extraction the urine must be strongly acidified with hydrochloric or sulfuric acid and either boiled for a short time or allowed to stand at room temperature for one or two weeks. The methods suitable for the working of human urine can be applied to mare urine only after extensive revision, and various new methods have been developed. For the initial removal of the hormone from the acid-hydrolyzed urine, Doisy and his collaborators³³ developed a process in which sodium benzoate is first added. The precipitate of finely divided benzoic acid carries down nearly all of the hormone, either by adsorption or by coagulating the sparingly soluble hydroxyketone. In the remainder of the process use is made of some of the purifications developed by the St. Louis group for the extraction from human urine and the final isolation is through the semicarbazone. By this method it is possible to obtain about 0.5 g. of the pure hormone from 19 liters of mare urine in 10 hours of working time. Another rapid method has been developed by Beall and Marrian.³⁴ Toluene is employed for the initial extraction and, after various partitions between organic solvents and alkali, the oestrone is precipitated as a mercury complex from an alkaline solution by the addition of mercuric chloride and ammonia. After acid hydrolysis of the mercury complex the hormone is obtained in a highly concentrated condition. Since it is the carbonyl group which is responsible for the formation of the complex, the other oestrogenic ketones present in mare's urine (see below) are precipitated along with oestrone.

Reputedly highly satisfactory is the method of isolation employed by Girard but not yet fully described in the scientific literature. This investigator discovered in trimethylaminoaceto-hydrazide hydrochloride, $(\text{CH}_3)_3\text{NCH}_2\text{CONHNH}_2$, a remarkable reagent for the isolation and puri-

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fication of ketones.³⁵ The substance reacts very readily with ketones to form water-soluble derivatives from which the purified ketones can

³² Zondek, *Arkiv Kemi, Mineral Geol.* 11B, No 21 (1933) [*Chem. Abs.*, 28, 3110 (1934)].

³³ Curtus, *J. Biol. Chem.*, 100, XXXIII (1933); Curtus, MacCorquodale, Thayer and Dowsy, *Ibid.*, 107, 101 (1934).

³⁴ Beall and Marrian, *J. Soc. Chem. Ind.*, 53, 30⁰T (1934).

³⁵ Girard and Sandulesco, *Brit. Pat. Appl.* 6640, March (1934).

be recovered with ease. To separate oestrone and other ketonic substances from a crude hormone oil a solution of the material in glacial acetic acid is warmed with Girard's reagent for a short time and poured into water. Inert oils and colored impurities are removed by extraction of the aqueous solution with ether, and the clarified solution of the hydrazone derivative is warmed with a slight excess of dilute acid. The ketonic substances are liberated and can be extracted with ether.³⁶

A source of oestrogenic material still better than the urine of pregnant mares was discovered by Zondek³⁷ as the result of further investigations of the occurrence of the hormone. Although oestrin plays a most important part in the physiology of the female organism, the occurrence, surprisingly enough, is not limited to the ovary, the placenta, and the urine of pregnancy. It appears from the work of the above-named investigator that the hormone is present in astonishingly large amounts in the genital glands and in the excretions of certain male mammals. The testis of the horse is the richest tissue known containing oestrogenic hormone. From the two testes of a stallion, together weighing 350 g., Zondek obtained an alcoholic extract containing 23,100 m. u. of material, which is about 300 times as great as that of both ovaries of a sexually mature mare. The average assay of stallion urine indicated a hormone content of 170,000 m. u. per liter. The identity of the oestrus-producing principle in stallion's urine with that obtained from female urines has been established fully by the isolation of pure, crystalline oestrone from this source.³⁸

The high hormone content of the urine of the male animal can be appreciated by comparing the values given in the accompanying table. The

HORMONE CONTENT ACCORDING TO ZONDEK (1934)

	Per liter (m. u.)	Per diem (m. u.)
Stallion	170,000	1,700,000
Mare	200	2,000
Pregnant mare	100,000	1,000,000
Sexually mature woman ..	70	170
Pregnant woman	10,000	15,000

stallion excretes nearly twice as much oestrin as the pregnant mare and an inordinately greater amount than the non-pregnant female animal. In a year the stallion produces about 62 g. of hormone. The paradox of a higher excretion of the female sex hormone by the male than by the female animal has been found only with equines (horse, zebra, ass, kiang).

³⁶ Other processes for the isolation of the hormones from urine include: extraction with molten stearic acid [Ger. Patent 610,237 (1935)], precipitation with carbonyl reagents [Brit. Patent 421,030 (1934)], and salting out with ammonium sulfate [U.S. Patent 2,001,256 (1935)]. See also Ger. Patent 618,165 (1935); Brit. Patent 432,435 (1935).

³⁷ Zondek, *Aritia Kami, Mineral. Geol.*, 11B, No. 24 (1933); *Nature*, 133, 200, 404 (1934).

³⁸ Doulefeu and Ferrari, *Z. physiol. Chem.*, 226, 192 (1934); *Compt. rend. soc. biol.*, 118, 538 (1935); Häumer, *Helv. Chim. Acta*, 17, 531 (1934).

For the bull, for example, the assay indicated only 330 m. u. per liter of urine.

According to Cartland *et al.*,¹⁸ stallion urine is superior to the urine of pregnant mares as a source of the hormone because it yields oestrone in a readily purified form relatively free from closely related substances which interfere in the case of mare urine. These investigators were able to obtain about 60% of the total activity of the original urine as crystalline oestrone. The urine was covered with one-third its volume of butanol, acidified strongly, and refluxed for four hours. The aqueous layer was extracted twice with butanol at room temperature by shaking and the combined butanol solutions were extracted with dilute soda solution and concentrated in vacuum under nitrogen. Butenandt's procedure was largely employed for the further enrichment. A mixture of urine from various stallions assayed 38,000 rat units per liter and yielded 16 mg. of crystalline oestrone (nearly pure) per liter. It is interesting that urine from a 2 year-old colt was found to contain no oestrogenic material.

Another surprising discovery is that oestrin occurs in the vegetable kingdom. It has been observed³⁹ that extracts from various flowers, as well as from certain lower animals and bituminous substances, possess definite oestrogenic activity, but this does not constitute a proof of the identity of the active principle in each case, particularly in view of certain observations to be recorded in a later section. In two cases, however, definite proof has been supplied by the chemical investigation of the active agent. Butenandt and Jacobi⁴⁰ succeeded in isolating pure oestrone (18 mg.) from a palm kernel extract (50 kg.), and Skarzynski⁴¹ obtained from female willow flowers (65 kg.) a crystallizate (7 mg.) of pure oestriol, the hormone hydrate (see below). The substances isolated from the plants produce the typical oestrous response in castrated animals. Whether or not the sex hormones play a part in plant biology is still a matter of uncertainty. Schoeller and Goebel⁴² observed that hormone preparations isolated from mammals accelerated the germination and growth of hyacinths and of certain other plants, but, although there have been some reports⁴³ of similar findings, most investigators⁴⁴ have obtained entirely negative results or have attributed observations similar to those of Schoeller and Goebel to the presence of impurities in the

¹⁸ Aschheim and Hohlweg, *Deut. med. Wochschr.*, 59, 12 (1933).

⁴⁰ Butenandt and H. Jacobi, *Z. physiol. Chem.*, 218, 104 (1933).

⁴¹ Skarzynski, *Bull. intern. acad. polonaise, Classe sci. math. nat.*, B11, 347 (1933). [*Chem. Abs.*, 28, 4755 (1934)].

⁴² Schoeller and Goebel, *Biochem. Z.*, 240, 1 (1931); 251, 223 (1932), 278, 298 (1935).

⁴³ Janot, *Compt. rend.*, 198, 1175 (1934); Scharrer and Schropp, *Chem. Abs.*, 28, 203d (1934); Choudard, *Compt. rend. soc. sci.*, 117, 1180 (1934).

⁴⁴ Virtanen, v. Hauser and Saastamainen, *Biochem. Z.*, 272, 32 (1934); Janot, *Compt. rend.*, 200, 1238 (1935); Harder and Stormer, *Jahrb. wiss. Botan.*, 80, 1, (1934); 81, 383 (1935); *Biochem. Z.*, 280, 176 (1935).

ficiently great to permit a nearly quantitative separation.⁵³ Oestriol alone is extracted by 0.1N sodium hydroxide solution from an ethereal solution of the mixture, and oestrone then can be extracted with 1N alkali. The method of Doisy yields about 0.3 mg. of oestrone and 1.3 mg. of oestriol per liter of human pregnancy urine.⁵⁴

The most generally accepted name for the follicular hormone hydrate is "oestriol" (Adam, *et al.*²⁷), but the substance is known also as "theelol" (Doisy) and as "Marrian crystals." Oestriol is not easily separated

PROPERTIES OF OESTRIOL

Formula	M p (corr.)	$[\alpha]_D$	Dissociation constant	Physiol. activity, m. u.	Triacetate, m. p.	Methyl ether, m. p.
$C_{18}H_{24}O_3$	280°	+ 30°	0.77×10^{-9}	per g 75,000	127°	159°

from traces of oestrone by crystallization, but the ketonic substance is easily removed as the sparingly soluble semicarbazone,⁵⁵ and samples so purified show a constant bio-assay. The oestrogenic potency of the hydrate is only about one one-hundredth that of the ketonic hormone, and its action is somewhat protracted. It is possible that the physiological activity is manifested only after the substance has been partially converted into the more active hormone by dehydration in the organism, but there is no evidence in support of this view and it does not appear very plausible. The triacetate is about 10 times as active as oestriol. In contrast to oestrone, which has little power to penetrate the intestinal walls, oestriol is only 2-3 times less active by the digestive route than when injected.

Other Oestrogenic Hormones. Girard and his collaborators⁵⁶ at Paris isolated from the urine of pregnant mares the interesting series of unsaturated hydroxy ketones whose properties are recorded in the table, page 202. Making use of the special ketone-reagent (page 197), the bulk of the oestrogenic material from some 52,000 kg. of urine was collected and carefully fractionated by distribution methods and crystallizations. Particular use was made of the optical activity of the fractions in conducting the purification of the three new compounds. The isomers equilin and hippulin contain two atoms of hydrogen less than

⁵³ S. L. Cohen and Marrian, *Biochem. J.*, **28**, 1603 (1934).

⁵⁴ Doisy and Thayer, *J. Biol. Chem.*, **91**, 641 (1931).

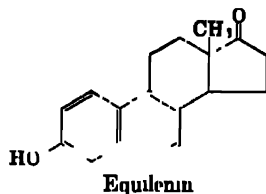
⁵⁵ Butenandt and Störmer, *Z. physiol. Chem.*, **208**, 120 (1932); Störmer, Dissertation, Göttingen (1933).

⁵⁶ Girard, Sandulesco, Fridenson and Rutgers, *Compt. rend.*, **194**, 909 (1932); Girard, Sandulesco, Fridenson, Gaudelroy and Rutgers, *ibid.*, **194**, 1020 (1932); Girard, Sandulesco and Rutgers, *ibid.*, **195**, 981 (1933); Sandulesco, Truong and Girard, *ibid.*, **196**, 137 (1933); Girard, *Bull. soc. chim. biol.*, **18**, 563 (1933).

HORMONES FROM MARE URINE

Substance	Formula	M p. (corr.)	$[\alpha]_D$	Physiol. activity (approx.), m. u.	Double bonds
Equilin	$C_{18}H_{20}O_2$	238-240°	+ 308°	1,300,000 ⁵⁷	4
Hippulin	$C_{18}H_{20}O_2$	233°	+ 128°	1,300,000	4
Equilenin	$C_{18}H_{18}O_2$	258-259°	+ 87°	600,000	5

oestrone and they possess one alicyclic double bond in addition to those present in a benzene nucleus, for each substance absorbs one mole of bromine. Equilenin has the properties of a naphthalene derivative (see formula) and it forms a very characteristic and stable picrate by means



of which the separation from oestrone and the other hormones is easily accomplished. Oestrone forms an easily dissociable semi-picrate. The structure of equilenin has been established by degradations which will be described in a later section.

Girard developed a sensitive colorimetric test for the ketonic naphthol based upon the conversion of the hormone into an amorphous red material when it is heated in the presence of air. All samples of oestrone from mare's urine were found to contain traces of equilenin, but no equilenin has been detected in human urine. The substance appears in mare's urine only after about the 175th day of pregnancy, and it becomes very abundant during the last months of the period of burden. As the amount of excreted equilenin increases the quantity of oestrone in the urine shows a considerable decrease. The relationship is very interesting, for it suggests that, as pregnancy progresses, oestrone is partially dehydrogenated in the animal organism to the less active equilenin.

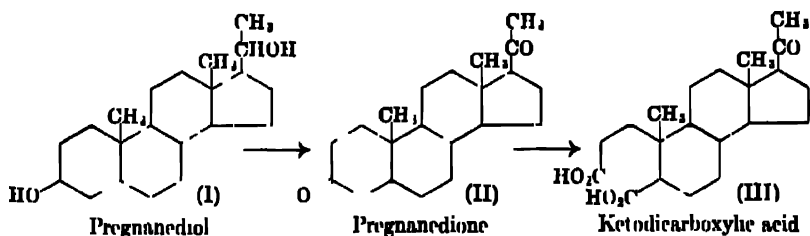
Pregnanediol. Searching for the follicular hormone, Marrian⁵⁸ in 1929 isolated a substance melting at 233-235° and possessing no oestro-

⁵⁷ More recently David and de Jongh [*Biochem. J.*, 29, 371 (1935)] found for equilin an activity one-third greater than that of oestrone by the spayed rat method, while with spayed mice Dirscherl and Hanusch [*Z. physiol. Chem.*, 233, 13 (1935)] noted equal activities. Cardland and R. K. Meyer [*J. Biol. Chem.*, 112, b (1935)] found equilin 75% as active (spayed rats) as oestrone when the solution injected contained 0.5% of sodium carbonate and only 30% as active when the soda was omitted.

⁵⁸ Marrian, *Biochem. J.*, 23, 1090 (1929).

genic activity. Butenandt⁸⁹ had observed this inactive companion substance at about the same time and soon published an account of the chemical characterization made possible by the accumulation of nearly 2 g. of the material from the residues remaining from the extraction of oestrone from 1000-2000 liters of urine. As Marrian had suspected, the substance contains more carbon atoms than the follicular hormone and the formula is $C_{27}H_{48}O_2$. By the observation that the primary oxidation product is a diketone, Butenandt established the presence of two secondary alcoholic groups, and the name which he adopted for the substance incorporates this feature of the structure. Pregnanediol proved to be a completely saturated substance and the composition indicated the presence of four hydrogenated rings in the molecule. This suggested a relationship to the bile acids, and another striking resemblance was found in the further oxidation of the diketone for, just as a bile acid yields a bilianic acid by the opening of one of the rings, pregnanedione gave a ketodicarboxylic acid. This located one of the two original oxygen atoms in a hydrogenated ring, and the other was found to be in the side chain, $-\text{CH}(\text{OH})\text{CH}_2$, by the observation that pregnanediol, pregnanedione, and the ketodicarboxylic acid all give positive iodoform reactions.

With this information, and on the assumption of a biogenetic relationship to sterols and bile acids, Butenandt suggested as a working hypothesis a structural formula based on the cholane formula accepted at the time (1930), and in the following year⁹⁰ he proceeded to prove the correctness of these inferences. His formulation requires no more than translation in terms of the modern cholane structure (I-III). Like lithobilianic acid, the ketodicarboxylic acid (III) was found to lose carbon dioxide on



pyrolysis and form a diketone, indicating that the nuclear hydroxyl group is located in a six-membered ring. This further similarity to the reactions of the bile acids was so suggestive that when it was found that pregnanedione can be reduced by the Clemmensen method to a saturated hydrocarbon, pregnane, it seemed reasonable to attack the problem from the other direction and to investigate the possibility of obtaining preg-

⁸⁹ Butenandt, *Ber.*, 63, 659 (1930).

⁹⁰ *Idem*, *ibid.*, 64, 2529 (1931).

genetic relationships until Hartmann and Locher⁶¹ discovered the presence of the stereoisomer, *allopregnanediol*, in pregnancy urine. The two inactive alcohols occur together in the neutral fraction which remains after the extraction of oestrone with alkali. The configuration (cholestane series) was established by comparison of the diketone obtained on oxidation with a substance which has been fully characterized in the course of the work on the corpus luteum hormone.

Still another saturated alcohol has been isolated from the urine of pregnant mares by Haslewood, Marrian and Smith,⁶² and the properties of the new substance are recorded in the accompanying table. The compound contains one hydroxyl group more than the other inactive compounds and it probably is a trihydroxy derivative of pregnane or of *allopregnanediol*. The location of the extra hydroxyl group is a matter of considerable interest, for this may reveal the identity of the precursor of the substance and its place in the series of metabolic changes.

INACTIVE ALCOHOLS

Compound	Formula	M p	$[\alpha]_D$	Acetate, m. p.	Acetate, $[\alpha]_D$
Pregnanediol . . .	$C_{21}H_{36}O_2$	235°	..	183°	+ 35.2°
<i>Allopregnanediol</i> . .	$C_{21}H_{36}O_2$	248°	...	142°	+ 18.8°
Saturated alcohol...	$C_{21}H_{38}O_1$	301°	-44°	168°	...

There have been some reports of the isolation from fat-soluble fractions of urine of compounds not related to the hormones. Marrian and Beall⁶³ obtained from ether-soluble phenolic fractions from the urine of mares and of stallions a non-oestrogenic substance equol ($C_{14}H_{14}O_1$), which they consider to be a chromane or coumarane. Hart and Northup⁶⁴ isolated and identified heptacosane, $C_{27}H_{56}$, from aged pregnancy urine, and they obtained some evidence of the presence of pentacosane. The hydrocarbons were obtained by adsorption on fuller's earth. No similar compounds were found in male urine or in non-pregnant female urine.

The Structure of Oestrone. Butenandt's brilliant work on pregnanediol was of no immediate assistance in determining the structures of the physiologically active hormones because no chemical relationship between these substances and the inactive pregnanediol had been established at the time when the chemical investigation of oestrone became the subject of active inquiry. Indeed the opinion was rather generally held that no

⁶¹ Hartmann and Locher, *Helv. Chim. Acta*, **18**, 100 (1935)

⁶² Haslewood, Marrian and E. R. Smith, *Biochem. J.*, **28**, 1316 (1934)

⁶³ Marrian and Beall, *ibid.*, **20**, 1546 (1935)

⁶⁴ Hart and Northup, *J. Am. Chem. Soc.*, **57**, 2720 (1935)

such connection existed, and work on the hormone was undertaken as a separate problem. As in the case of other phenanthrene derivatives, notably retene, the combustion of oestrone presented particular difficulties, but reliable analyses of the substance itself and of various derivatives eventually were achieved by both the German⁶⁵ and the American⁶⁶ investigators, and the results indicated clearly the empirical formula $C_{18}H_{22}O_2$. One oxygen atom was recognized as ketonic by the preparation of an oxime (Butenandt), the other was characterized as constituting a weakly acidic phenolic group (Butenandt, Doisy, Marrian), and it was soon generally agreed that the hormone is a hydroxy ketone containing one benzenoid ring. Early in 1932, Bernal,⁶⁷ whose work on the sterols had furnished the inspiration for the drastic revision of the old cholane formula, reported the results of an X-ray and crystallographic study of the hormone which attracted considerable attention. Bernal noted that the molecules do not form a double layer structure, as in the case of the long-chain alcohols having a polar group at but one end of the molecule, and he correctly concluded that the hormone consists of a long molecule with the hydroxyl at one end and the carbonyl group at the other.

The preliminary calculations of the molecular dimensions, on the other hand, unfortunately were in error, for they indicated that oestrone probably contained as the fundamental element a three-ring, rather than a four-ring nucleus. Instead of a perhydrocyclopentenophenanthrene, or sterol-like, ring system, a reduced phenanthrene nucleus was indicated. Pregnanediol was declared by Bernal to be similar to the sterols and entirely unlike oestrone. Studies by Adam and Danielli⁶⁸ of the monomolecular films formed by some of the hormone derivatives pointed in the same direction, although the distinction between the alternate ring systems was less definite. The composition of the hormone was such as to indicate either a four-ring system with one benzenoid nucleus, or a three-ring system with one benzene ring and one additional double bond, and the latter hypothesis, given prominence by Bernal's preliminary analysis of the X-ray data, appeared to offer a ready interpretation for some of Butenandt's early observations.⁶⁹ Doisy⁶⁸ thought that an ethylenic double bond was indicated by the ready reaction of oestrone with bromine, but Marrian and Haslewood⁷⁰ soon showed that the substance formed is not an addition product but simply a product of substitution in the phenolic ring.

⁶⁵ Butenandt, *Z. physiol. Chem.*, **191**, 140 (1930); Butenandt and Hildebrandt, *ibid.*, **199**, 243 (1931).

⁶⁶ Thayer, Levin and Dixon, *J. Biol. Chem.*, **91**, 791 (1931).

⁶⁷ Bernal, *Chemistry and Industry*, **51**, 239 (1932).

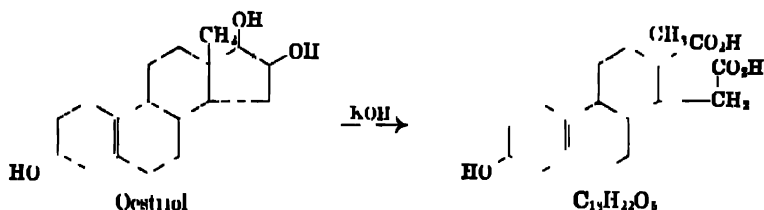
⁶⁸ Adam, Danielli, Haslewood and Marrian, *ibid.*, **51**, 259 (1932); *Biochem. J.*, **26**, 1233 (1932). See also Danielli, Marrian and Haslewood, *ibid.*, **27**, 311 (1933).

⁶⁹ Butenandt, Störmer and U. Westphal, *Z. physiol. Chem.*, **208**, 149 (1932).

⁷⁰ Marrian and Haslewood, *Lancet*, Aug. 6 (1932), *J. Soc. Chem. Ind.*, **51**, 277T (1932).

In order to settle the question of the degree of saturation of the hormone molecule, Butenandt undertook a study of the catalytic hydrogenation and the molecular refraction of oestrone,⁷¹ and in the autumn of 1932 he reported results⁷² which pointed clearly to the presence of only three double bonds. This left as the only possibility a four-ring system, and the suggestion of a structural similarity to the sterols and to pregnanediol led Butenandt to visualize the structure which is now known to be correct. The polar groups were placed at opposite ends of the molecule, following Bernal's evidence, the hydroxyl group was assumed to occupy the same position as in cholesterol or lithocholic acid, and the ketonic oxygen atom was located at the position corresponding to the point of attachment of the side chains of the sterols and the bile acids. Essentially the same formula was advanced independently by Marrian and Haslewood,⁷³ and it was not long before the deductions were shown to be correct.

A route to a successful degradation was opened by the independent observations of Marrian and Haslewood⁷³ and of the Doisy group⁷⁴ concerning the action of fused alkali on oestriol, whose relationship to oestrone had been established by Butenandt's conversion of the one substance into the other. The fusion product was a phenol dicarboxylic acid having all of the original carbon atoms, and it was inferred that the two alcoholic groups of oestriol occupy adjacent positions and that the ring is cleaved between these two positions. The dibasic acid forms an



anhydride and not a ketone when heated with acetic anhydride and, in view of the evidence that the original ring is at the end of the molecule, this can be taken as an indication of a five-membered ring in the hormone structure.

In 1933 Butenandt⁷⁵ carried the degradation two steps further. The phenol dicarboxylic acid was subjected to dehydrogenation with selenium and there was formed, in a particularly smooth reaction, a dimethylphenanthrol. Distillation with zinc dust yielded 1,2-dimethylphenan-

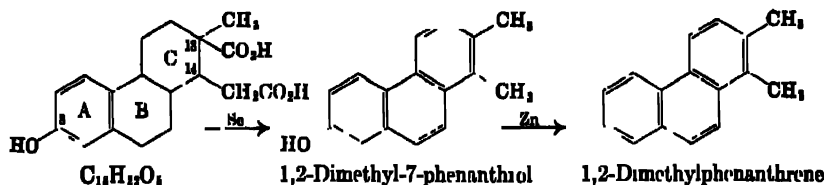
⁷¹ For the final results, see Butenandt and U. Westphal, *Z. physiol. Chem.*, **223**, 147 (1934).

⁷² Butenandt, *Nature*, **130**, 238 (1932), *Z. anorg. Chem.*, **48**, 635 (1932).

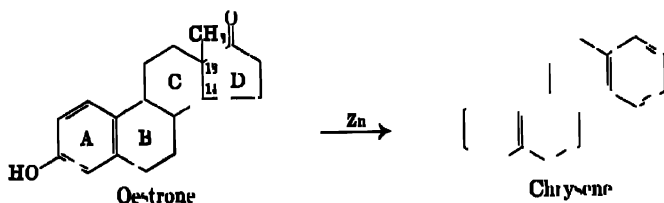
⁷³ Marrian and Haslewood, *J. Soc. Chem. Ind.*, **51**, 277T (1932).

⁷⁴ MacCotquodale, Thayer and Drury, *J. Biol. Chem.*, **99**, 327 (1933).

⁷⁵ Butenandt, Weidlich and H. Thompson, *Ber.*, **66**, 601 (1933).



threne, a previously unknown hydrocarbon which was synthesized for comparison from naphthalene and methyl succinic anhydride by the general method developed by Haworth (page 71). This proved conclusively the presence in the original hormone of three six-membered rings (A, B, and C) in the phenanthrene arrangement, and, since a further, five-membered ring was already established, the relation to the sterol structure was clearly indicated. A confirmation of this conclusion was found in the isolation of the identical dimethylphenanthrene from the dehydrogenation of actiobilanic acid, a reaction discussed in the preceding chapter. Since one of the methyl groups of the dimethylphenanthriol obviously arises from the decarboxylation of the acetic acid residue of the phenol dicarboxylic acid, the original five-ring must be joined to ring C at either C_{13} or C_{14} . Some evidence that the five-membered ring is attached to both of these positions and that the angular methyl group is located at C_{13} was afforded by the observation⁷⁶ that oestrone yields a small amount of chrysene when distilled over zinc dust. Because of the regularity with



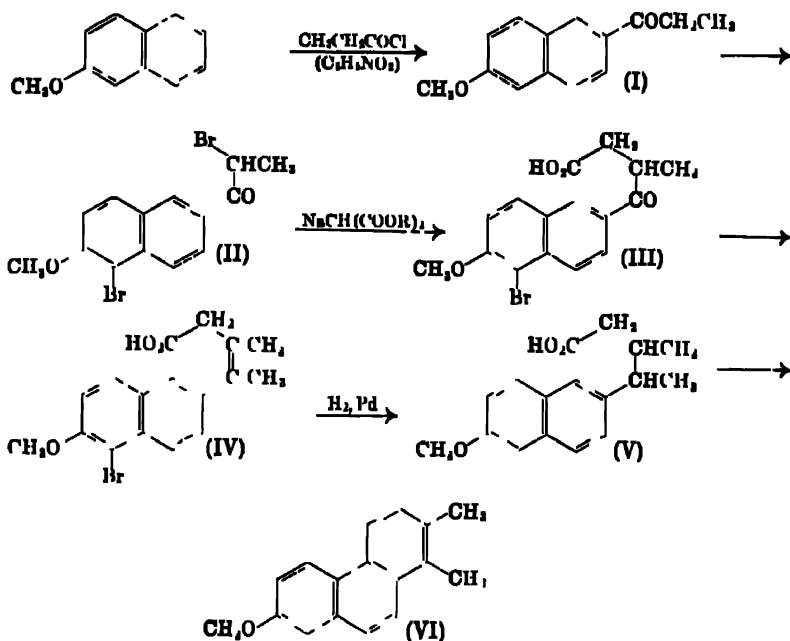
which this type of ring enlargement has been observed in the sterol and bile acid series, some reliance can be placed in this otherwise dubious evidence. A still more certain indication that the five-membered ring is in the same position as in the sterols was furnished by the observation of Danielli⁷⁷ that the value found for the cross-sectional area of the hormone molecule by unimolecular film measurements⁷⁸ agrees closely with the minimal area calculated for a structure of the sterol type but not with the calculated areas for other possible structures. The experimental value is 34 sq. Å, with a possible error not greater than 2 sq. Å; the calculated minimal area is 33 sq. Å.

⁷⁶ Butenandt and H. Thompson, *Ber.*, **67**, 140 (1934).

⁷⁷ Danielli, *J. Am. Chem. Soc.*, **56**, 746 (1934).

⁷⁸ Danielli, Marrian and Haelewood, *Biochem. J.*, **27**, 311 (1933).

The location of the hydroxyl group, and consequently the positions of the three benzenoid double bonds which confer upon it phenolic acidity, could only be inferred on biogenetic grounds until a proof was forthcoming in 1934 from the laboratories of Haworth and of Cook. Haworth⁷⁹ worked out a synthesis of 1,2-dimethyl-7-methoxyphenanthrene for comparison with the methyl ether of Butenandt's degradation product. An unusual orientation in the Friedel and Crafts reaction furnished a suitable starting material, I. In brominating the ketone a second bromine



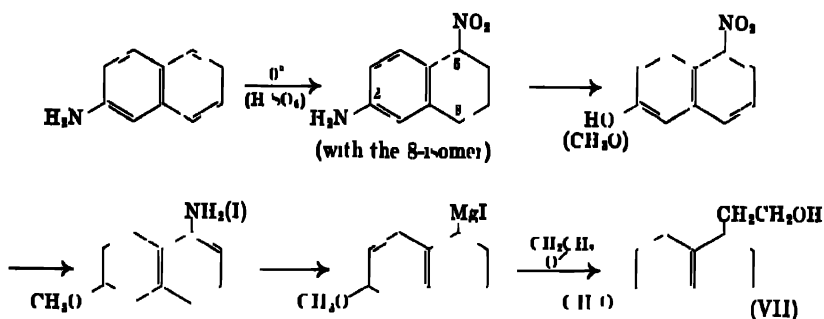
atom entered the nucleus, but this was eliminated later by hydrogenation. A malonic ester condensation followed by hydrolysis afforded the acid III, and a Grignard reaction with the ester of III provided a means of acquiring the second methyl group desired. Hydrogenation to V, followed by ring closure through the acid chloride (with stannic chloride), reduction and dehydrogenation with selenium gave the desired product VI. A direct comparison established the identity with Butenandt's material, proving the location of the hydroxyl group and of the unsaturated ring in oestrone.

Cohen, Cook, Hewett and Girard⁸⁰ carried the evidence a step further, for they succeeded in synthesizing a substance not only having a hydroxyl group in the correct position in the phenanthrene nucleus, but containing

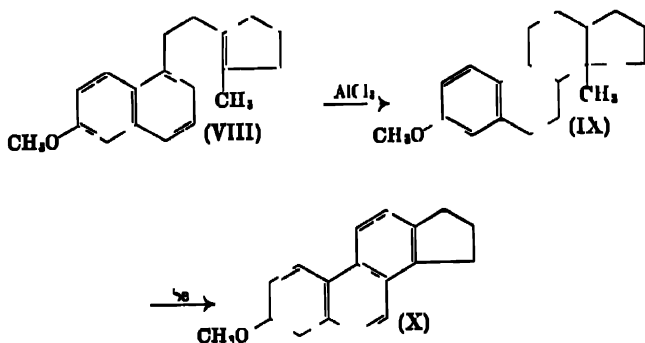
⁷⁹ R. D. Haworth and Sheldrick, *J. Chem. Soc.*, 964 (1934)

⁸⁰ A. Cohen, Cook, Hewett and Girard, *ibid.*, 653 (1934)

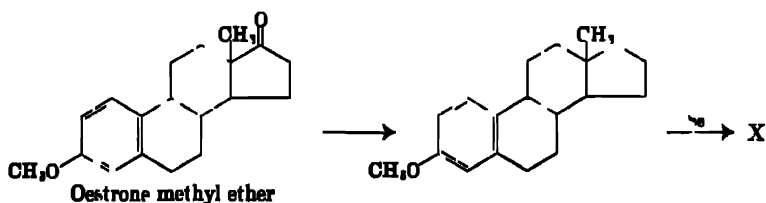
also the five-membered ring of the hormone. The most difficult task was that of obtaining the desired heteronuclear disubstituted naphthalene derivative VII for use in the Perlman-Davidson-Bogert synthesis, but a method was developed for the preparation of the substance from β -naphthylamine through the following sequence of reactions.



The Grignard reagent obtained through the chloride of the alcohol (VII) was condensed with 2-methylcyclopentanone, affording, after dehydration of the resulting carbinol, the unsaturated substance VIII. The purpose of

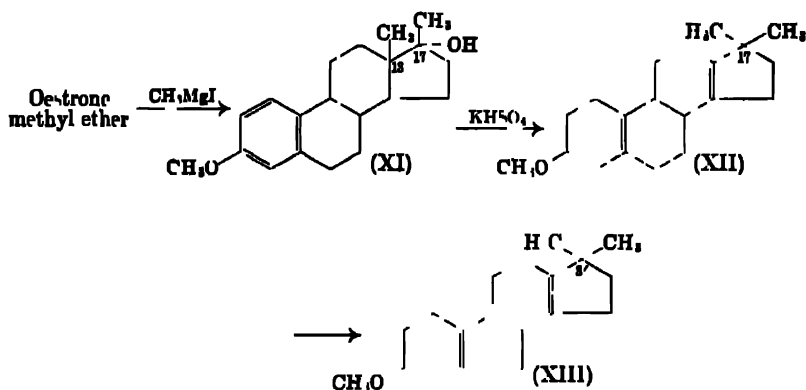


the methyl group was to hinder the formation of isomeric spurs in the cyclization of VIII, and the group in question was eliminated easily in the dehydrogenation of IX. The synthetic route was a long one and 900 g of β -naphthylamine yielded only 0.36 g of the desired 7-methoxy-1,2-cyclopentenophenanthrene, X, but this substance was found to be identical with the material obtained from oestrone by methylation, reduction by the



Wolff-Kishner method, and dehydrogenation.⁸¹ The same substance (X) was obtained by a similar degradation of equilenin, proving the correspondence of the ring system and of the hydroxyl group of this hormone with the oestrone molecule.

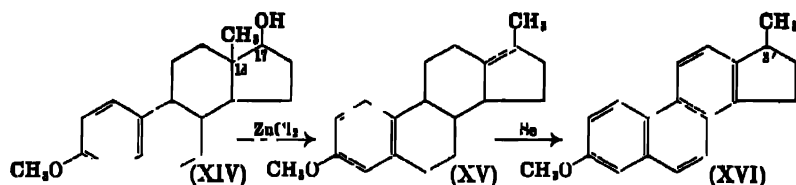
With the object of defining the position of the carbonyl group in oestrone, Cohen, Cook and Hewett⁸² submitted its methyl ether to reaction with methyl magnesium iodide, dehydrated the resulting carbinol, hydrogenated the ethylenic linkage thus produced, and dehydrogenated the saturated product with selenium. It was expected that the methyl group introduced in the Grignard reaction would appear in the product of dehydrogenation at a position corresponding to that of the original carbonyl group, but the substance was not identical with the 1'-, the 2'-, or the 3'-methyl derivative of 7-methoxy-1,2-cyclopentenophenanthrene, all which were prepared synthetically for comparison. Instead, the compound was found to be identical with synthetic 7-methoxy-3',3'-dimethyl-1,2-cyclopentenophenanthrene (XIII), and it was concluded that a molecular rearrangement occurs in the course of the dehydration of the carbinol (XI). According



to formula XI the Grignard product contains a carbinol group adjacent to a quaternary carbon atom (C_{13}) and consequently it is of a type with which dehydration is attended by group migration. Evidently the angular methyl group at C_{13} migrates in the course of the dehydration to position 17 in the five-membered ring. A proof of the correctness of this interpretation was secured by the observation that a methyl migration also occurs in the dehydration of the phenolic methyl ether of dihydro oestrone, XIV, which also has a carbinol group joined to a quaternary carbon atom. The dehydration product, XV (or a bond-isomer), yields 7-methoxy-3'-methyl-1,2-cyclopentenophenanthrene (XVI) on dehydro-

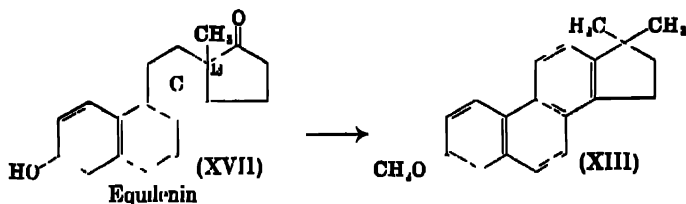
⁸¹ Cook and Girard, *Nature*, 133, 377 (1934)

⁸² A. Cohen, Cook and Hewett, *J. Chem. Soc.*, 445 (1935)



genation. The methyl group appearing at position 3' in the final product is the angular methyl group of the original oestrone molecule, and the transformations supply a rigid proof that this is located at C₁₃ and that the keto group occupies the 17-position. Since the structures of the dehydrogenation products XIII and XVI are fixed by synthesis, and since in the case of XIII the possibility of a rearrangement in the treatment with selenium was excluded by the hydrogenation of XII prior to this treatment, no other formulations are consistent with the experimental facts.

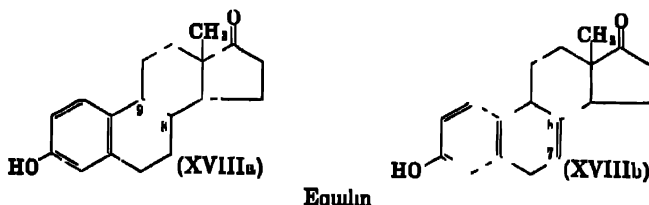
This work of the English investigators completes the evidence regarding the structure of oestrone. Two of the hormones isolated by Girard from mare's urine were also characterized further in the investigation of Cohen, Cook and Hewett, both equilenin and equilin being converted by the above methods into 7-methoxy-3',3'-dimethyl-1,2-cyclopentenophenanthrene. This establishes the nature of the ring system and the location of the hydroxyl, methyl, and carbonyl groups, as with oestrone, and in the case of equilenin the new evidence, coupled with the observation that the molecule contains a naphthalene nucleus (picrate formation), completely proves the structure of the hormone. Ring C clearly cannot be one of the two, connected aromatic rings because of the presence of the angular methyl group at C₁₃, and no structure is possible other than that indicated in formula XVII.



Equilin contains one aromatic ring and one additional nuclear double bond. Dirscherl⁸³ attempted to hydrogenate this double bond, with the expectation of obtaining oestrone or a stereoisomer, but found that the catalyst exerted a dehydrogenating action even in the presence of hydrogen. By heating equilin with palladium black at 80°, he was able to

⁸³ Dirscherl and Hamusch, *Z. physiol. Chem.*, 233, 13 (1935), 236, 131 (1935); Dirscherl, *Z. angew. Chem.*, 48, 309 (1935).

transform the hormone into a pure substance identical with equilenin. The tendency to undergo dehydrogenation indicates that the double bond is in the ring (B) adjacent to the aromatic ring (A), and Dirscherl noted that the resistance of the bond to hydrogenation favors the location C₅-C₆.



(as in apocholic acid, page 132). He observed on the other hand that the ultraviolet absorption curve of equilin is almost identical with that of oestrone, whereas a considerable difference would be expected if the ethylenic linkage were in a position of conjugation, as in XVIIIa. This evidence, which Cook and Roe⁸⁴ regard as conclusive, favors the formulation XVIIIb.

Oestradiol and Other Derivatives of Oestrone. Of the oestrogenic substances isolated from natural sources before 1935, oestrone was found to be the most potent in the Allen-Doisy test. It was discovered, however, at a fairly early date that the hormone is surpassed in activity by certain substances which can be obtained from it by chemical transformations. The most interesting and important derivative is the dihydro compound oestradiol, which Schwenk and Hildebrandt⁸⁵ first prepared in a somewhat impure condition (m p 168-170°) by the reduction of oestrone at the carbonyl group. A stereoisomeride was said to accompany the main reaction product. Apparently there is considerable variation in the results of assay depending upon the details of the test method employed, for oestradiol has been reported to be from two⁸⁶ to five⁸⁷ times as active as oestrone. Girard, Sandulesco and Fridenson⁸⁸ obtained the same substance (m p 174-175°, corr.) as the sole product of reduction with sodium and alcohol or by hydrogenation,⁸⁹ and according to a patent claim⁹⁰ oestradiol can be prepared by heating oestrone with cyclohexanol at 200° in the presence of a nickel catalyst.

Because of its high potency in comparison with any of the hormones

⁸⁴ Cook and Roe, *Chemistry and Industry*, 54, 501 (1935).

⁸⁵ Schwenk and Hildebrandt, *Naturwissenschaften*, 21, 177 (1933).

⁸⁶ David, *Acta Biologica Neerlandica*, 3, 160 (1933); David, de Jongh and Laqueur, *Arch intern pharmacodynamie*, 51, 137 (1935).

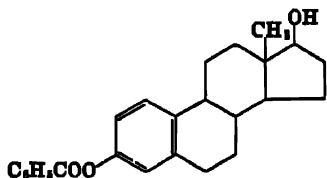
⁸⁷ Schoeller, Dohrm and Hohlweg, *Klin Wochschr*, 14, 526 (1935).

⁸⁸ Girard, Sandulesco and Fridenson, *Compt rend soc biol*, 112, 964 (1933).

⁸⁹ These investigators did not observe any appreciable increase in activity, probably because of a difference in the technique of assay.

⁹⁰ Schering-Kahlbaum A-G, Brit patent 429,747 (1935).

obtainable from urinary extracts, oestradiol has attracted considerable attention as a possible therapeutic agent, and the phenolic benzoate is regarded as a particularly valuable substance for clinical use. Studies of the physiological properties of various derivatives of oestrone had shown that the esters are characterized by a protracted and persistent action, and the same is true of the corresponding dihydro derivatives. A



Oestradiol monobenzoate

given effect can be produced with one large dose of oestrone benzoate, whereas with the hormone itself several injections of small doses would be required. Oestradiol monobenzoate, a substance which combines the high oestrogenic activity of the diol with the desirable properties of the esters, has been introduced into therapy under the name "Progynon B oleosum" (Butenandt).

The realization that oestrone is not as active, physiologically, as its dihydro derivative led workers in the field to suspect that even the most potent of the substances excreted in the urine may not be the true follicular hormone responsible for normal physiological changes in the body. The investigation of the constituents of ovarian tissue presented many difficulties, but in 1935 Doisy and co-workers⁹¹ completed the working of one and one-half tons of hog ovaries and reported the isolation of a minute quantity of an active substance as the *m*-bromobenzoyl derivative. On hydrolysis there was obtained a substance, m.p. 170-171°, which was found to be from four to eight times as potent as oestrone in adult castrate rats. A comparison with oestradiol proved the identity of the hormone with this compound. According to Doisy's assays, the greater part of oestrogenic activity of ovarian extracts is due to oestradiol, and consequently this substance is probably a genuine hormone of the body and the principal oestrus-producing agent of follicular fluid. Oestrone and the other oestrogenic substances excreted in the urine of females and males alike, now appear to be secondary products of minor importance to the organism, however great has been their value in advancing the problem and in therapy. The discovery of the principal ovarian follicular hormone followed a course which is remarkable and unique. Oestradiol was known, its structure had been established, and therapeutic uses

⁹¹ Doisy, MacCorquodale and Thayer, *Weekly Bull. St. Louis Med. Soc.*, 29, 435 (1935); MacCorquodale, Thayer and Doisy, *Proc. Soc. Exptl. Biol. Med.*, 32, 1182 (1935).

of the pure substance had been secured by patents, even before the difficult task of isolation from natural sources had been accomplished.

There have been some further reports of an increase in the physiological activity of urinary hormones following chemical modification of the structures. In analogy with oestradiol, David⁸² found that the diols obtained by the reduction of equilin and equilenin are approximately twice as active, and three times as active, respectively, as the hydroxy-ketones. Although the drastic modification of the oestrone structure resulting from the oxidative opening of the five-membered ring might be expected to destroy the special physiological activity, Doisy and co-workers⁸³ discovered certain oxidation products of the phenolic methyl ether of oestriol which are reported to be considerably more active than pure oestrone (7-8 fold increase!).

Oestrogenic substances have been used in therapy⁸⁴ chiefly in the treatment of menopausal vasomotor symptoms, amenorrhea, and gonorrheal vaginitis (in children). The most convenient method of administration is by mouth, although the amount of material required is several times the effective hypodermic dosage. At present, however, the oestrogenic preparations are too expensive to be generally employed in this way, and the applications have been limited. Opinions still differ regarding the advisability of some of the clinical uses of hormone preparations, and the possible dangers have not been fully explored.

Synthetic Substances Possessing Oestrogenic Activity. It is evident from the foregoing discussion that the hormone molecule is subject to considerable modification without loss of the ability to produce the oestrous response, and that in some cases the activity is increased. The characteristic physiological property is also shared to a greater or less degree by the three substances of the equilin group isolated by Girard, and by oestriol. There is some possibility that all of these substances suffer transformation into oestrone in the course of the tests and that their varying degree of potency is due to differences in the ease of conversion, but the weight of evidence favors the view that oestrogenic activity is not entirely specific. The most cogent argument is that substances of widely differing structural types have been found capable of inducing the changes characteristic of the oestrous state.

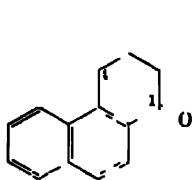
Even before the structure of oestrone had been completely established, and at a time when it was known with certainty only that the oestrus-producing hormone is a hydroxy ketone containing a reduced phenan-

⁸² David, *Acta Brera Norrland*, 4, 63 (1934)

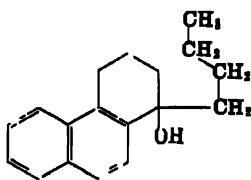
⁸³ MacCorquodale, Levin, Thayer and Doisy, *J. Biol. Chem.*, 101, 753 (1933)

⁸⁴ Novak, *J. Am. Med. Assoc.*, 104, 1815 (1935); C. Kaufmann, *Deut. med. Wochenschr.*, 61, 861 (1935).

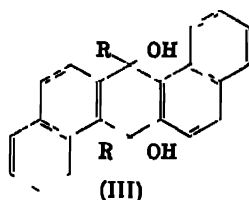
threne nucleus, Cook and Dodds⁸⁵ commenced an investigation of the possible oestrus-activity of synthetic compounds bearing some points of similarity in structure to the natural hormone. The discovery was soon made that the oestrogenic activity is amazingly unspecific and that the complicated oestrous change can be induced by a whole host of substances bearing little resemblance to the hormone. One of the simplest of these, 1-ketotetrahydrophenanthrene (I), produces the full oestrous response



(I)



(II)



(III)

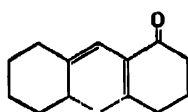
including uterine changes when a 100 mg. dose is administered in a single injection to a castrated rat. The action is somewhat protracted, but the oestrus is of very prolonged duration. The isomeric 4-ketotetrahydrophenanthrene is inactive and the *n*-butyl carbinol II is less active than the parent ketone (I). 1,2-Cyclopentenophenanthrene shows no activity. Several diols of the type III, obtained by the action of the Grignard reagent on 1,2,5,6-dibenzanthraquinone, are also active, and there is an interesting variation in the series with change in the molecular weight. The dimethyl compound is inactive in a 100 mg. dosage, the diethyl compound produces oestrus in doses of 1 mg., the di-*n*-propyl derivative is active in as small amounts as 0.025 mg. The lengthening of the side chains beyond this point results in a drop in the activity, the effective dose for the di-*n*-butyl compound being 0.1 mg. The di-*n*-amyl compound is inactive. The synthetic di-*n*-propyl diol is considerably more potent than oestriol although it falls far short of the activity of oestrone.

Another comparison between the synthetic substances and the natural hormone has been made with the use of rapons.⁸⁶ The subcutaneous injection of oestrone in large doses into the birds caused the plumage to change from male to female, and the same results were obtained with 1-ketotetrahydrophenanthrene and with di-*n*-butyl-dihydroxy-dihydro-1,2,5,6-dibenzanthracene.

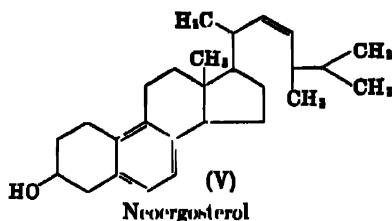
Compounds I, II, and III, listed above, all contain the phenanthrene nucleus, but this feature does not appear to be essential for oestrogenic activity for the ketone IV, containing a reduced anthracene grouping,

⁸⁵ Cook, Dodds and Hewett, *Nature*, **131**, 56 (1933); Cook and Dodds, *ibid.*, **131**, 205 (1933); Cook, Dodds, Hewett and Lawson, *Proc. Roy. Soc. (London)*, **B114**, 272 (1934).

⁸⁶ Cook, Dodds and Greenwood, *ibid.*, **B114**, 286 (1934).



(IV)



(V)

Neosterosterol

shows some pro-oestrus activity. Most of the sterols were found to be inactive, but some of the more unsaturated members of the series gave positive tests. This was true of ergosterol and vitamin D₂. Neosterosterol (V) which contains one aromatic ring and one double bond in the side chain, was the most oestrogenically active member of the group. From these and other observations, Cook and Dodds concluded that a certain degree of unsaturation is necessary and that the presence of an oxygen atom in the molecule, although not essential, usually is required for the attainment of the greatest potency.

A few hydrocarbons were found to possess at least weak oestrogenic activity and it is most interesting that the list includes the two carcinogenic compounds 1,2-benzpyrene and 5,6-cyclopenteno-1,2-benzanthracene. Although the hydrocarbons are active only in large (100 mg.) doses, they produce the full oestrous response in a certain proportion of the animals tested. The striking observation that these substances are both carcinogenic and oestrogenic raises the question of a possible connection between the phenomena of oestrus and malignancy. It is perhaps suggestive that the cellular proliferation characteristic of the oestrous state bears at least a superficial resemblance to the early proliferative stages of carcinoma.

Oestrogenic Hormones and the Cancer Problem. That the female sex hormones may be connected either directly or indirectly with the initiation of certain forms of malignant growth has been suggested by the results of investigations of the incidence of cancer in mice in relationship to oestrogenic substances. Notable contributions to the problem were made by Leo Loeb and his collaborators⁹⁷ long before the development of the chemistry of the hormones. In breeding experiments (1907-1919) Loeb found that different strains of mice can be developed in each of which the rate of incidence of spontaneous mammary cancer is approximately constant while from strain to strain it varies from nearly 0% to almost 100%. In some strains practically none of the mice develop tumors, while in others nearly all of the females are subject to cancer of the breast, the most frequent form of tumor found in this species. Males, even of high cancer rate strains, usually do not develop mammary tumors.

The cancer rate remains approximately constant in successive generations, and both the incidence of cancer and the age at which tumors appear are characteristic of each strain. A knowledge of these characteristics provides a basis for the investigation of the influence of other factors.

Using female mice of known high-incidence strains, Loeb found that removal of the ovaries at the age of 3-4 months led to a marked decrease in the incidence of cancer. Castration at a sufficiently early period reduced the cancer rate definitely to zero. Extirpation of the ovaries at later periods was less effective, and when performed at the age of 8-10 months the cancer rate was practically the same as with the untreated animals, although cancer appeared at a later age than in the non-castrated mice. These and other results strongly suggested that ovarian hormones, acting in cooperation with hereditary factors, influence the transformation of normal tissue of the mammary gland into cancerous tissue.

Seeking confirmation of this view, Loeb attempted to induce cancer formation by transplanting ovaries of female mice into spayed males belonging to high cancer rate strains, but with negative results. More successful experiments in this direction have been carried out with the use of pure hormone preparations. Lacassagne,⁹⁸ by giving weekly injections of crystalline oestrone benzoate dissolved in sesame oil to young mice, succeeded in producing mammary cancer in male mice in which it normally would not have appeared. Female mice generally showed a higher incidence of cancer following the treatment. In one strain of mice oestrone benzoate gave rise to no mammary tumors. It appears from this work that in certain strains of mice, but not in all, the prolonged administration of an oestrogenic substance leads to an increased disposition of the animal to develop cancer of the breast. Burrows⁹⁹ studied the effect of the prolonged application of oestrone in 0.01% benzene solution to the skin of stock mice and found that following the treatment, the development of mammary cancer among the male mice was very uncommon, probably because of a natural refractoriness. He observed, however, that the administration of oestrone to both male and female mice frequently was followed by cystic mastopathy, a condition regarded as a preparatory stage toward cancer. Synthetic oestrogenic substances gave similar results.

There are some preliminary indications from assays of the blood of cancer patients¹ and of tumor-bearing male mice² that there is an over-

⁹⁸ For an excellent review, see Leo Loeb, "Estrogenic Hormones and Carcinogenesis," *J. Am. Med. Assoc.*, 104, 1807 (1935).

⁹⁹ Lacassagne, *Compt. rend.*, 195, 630 (1932); Lacassagne and Nyks, *Compt. rend. soc. biol.*, 116, 844 (1934).

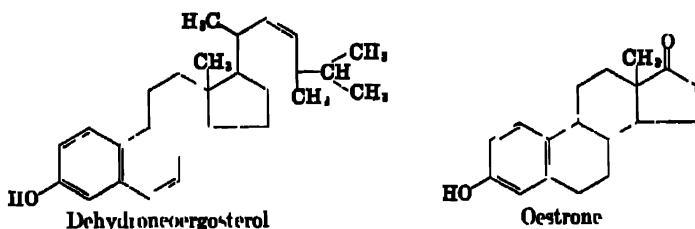
¹ Burrows, *British Journal of Surgery*, 23, 191 (1935).

² Dingemans, Freud, de Jongh and Laqueur, *Arch. Gynaekol.*, 141, 225 (1930).

³ Loewe, Raudenbush and Voss, *Biochem. Z.*, 249, 443 (1932).

production of oestrogenic material by the organism during malignancy, but this important point has not been definitely established.

The Synthetic Approach to the Hormone Structure. Active interest in the elaboration of methods for the synthesis of substances incorporating some or all of the structural features of the hormone molecule has been prompted by various considerations. Therapeutic uses have been indicated for the natural oestrogenic hormones, but the extraction of these substances from urine is tedious and costly and the yields at best are very small. It is quite possible that compounds of the oestrone group can be obtained by the degradation of sterols or bile acids, and a distinct advance in this direction is the conversion of ergosterol through neoergosterol into a naphtholic compound, dehydroneoergosterol (page 177). The ethylenic linkage in the side chain offers possibility for further degrada-

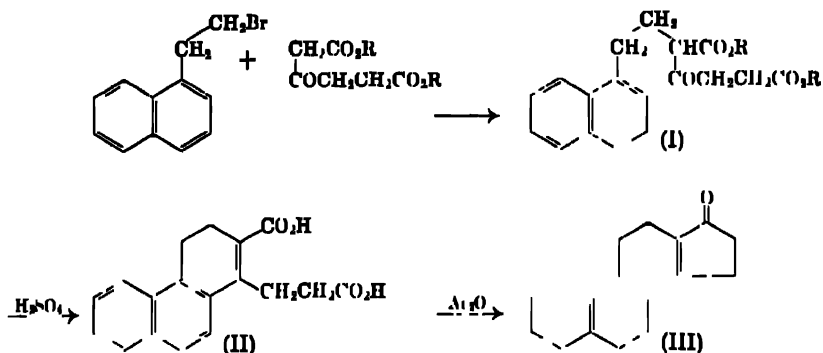


tion approaching the structure of oestrone. It seems questionable, however, if a lengthy series of transformations from such a costly starting material as ergosterol can offer advantages over methods of total synthesis. A synthetic method, moreover, would have greater flexibility and might afford comparison compounds of considerable interest in exploring further the relation-ship of oestrogenic activity to structure and in investigating possible pathological changes akin to cancer. It is an attractive feature of the synthetic approach that even though a plan may fall short of the final mark of duplicating the work of nature it may provide physiologically active substances of importance for purposes of biological experimentation and possibly of therapeutic value.

In the synthetic work which met with such brilliant success in elucidating the structures of the oestrogenic hormones the closest approach to the structure of oestrone was the synthesis by Cook and co-workers of 7-methoxy-1,2-cyclopentenophenanthrene (page 210). The free phenol obtained on hydrolysis has the ring system and the hydroxyl group of the hormone, but it lacks the angular methyl group and the carbonyl group associated with the five-membered ring and two more rings are aromatised than in the case of oestrone. According to the preliminary report, the synthetic phenol produces a strong oestrous response in spayed mice when injected in doses of 10 mg.

Work on the development of other synthetic methods is in progress in several different laboratories at the time of this writing, and in anticipation of rapid advances in the field it will be sufficient to indicate briefly some of the lines of attack suggested in investigations which are still incomplete.

Bardhan, in an investigation which thus far has been reported only in a preliminary communication,³ condensed β -(1-naphthyl)-ethyl bromide with the sodium derivative of methyl β -ketoadipate and cyclized the



keto ester I with sulfuric acid. On distillation of the dibasic acid II with acetic anhydride there was produced a substance having the composition and presumably the structure of the ketone III. Oestrogenic activity of this compound is indicated. The plan of cyclization employed in the synthesis is essentially that discovered by Bougault⁴ for the preparation of indenes and adapted by von Auwers and Moller⁵ for the closing of six-membered rings. Bougault cyclized the keto ester IV with sulfuric acid and obtained the diester of indene-1,2-dicarboxylic acid V. A ring closure of much the same type is involved in one modification of the general



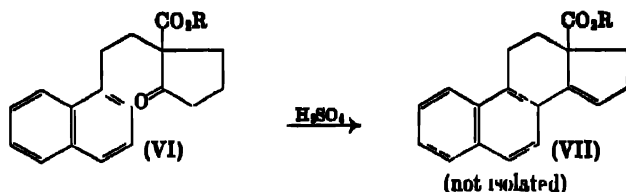
Bardhan-Sengupta phenanthrene synthesis. The regular method for cyclizing the keto ester VI consists in removing the ester group by hydrolysis, reducing the ketone, and effecting a cyclodehydration of the alcohol (page 161). It will be recalled, however, that Ruzicka⁶ found that VI is capable of undergoing direct cyclization, the final product of the action

³ Bardhan, *Nature*, 134, 217 (1934)

⁴ Bougault, *Compt. rend.*, 159, 745 (1915)

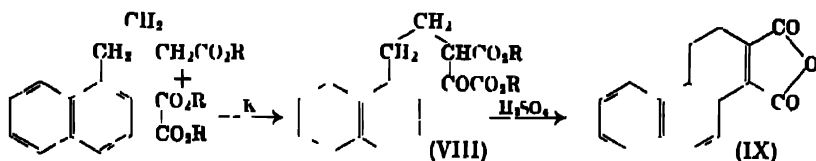
⁵ von Auwers and K. Moller, *J. prakt. Chem.*, 100, 124 (1925)

⁶ Ruzicka, Ehrmann, Goldberg and Hübel, *Helv. Chim. Acta*, 16, 833 (1933)



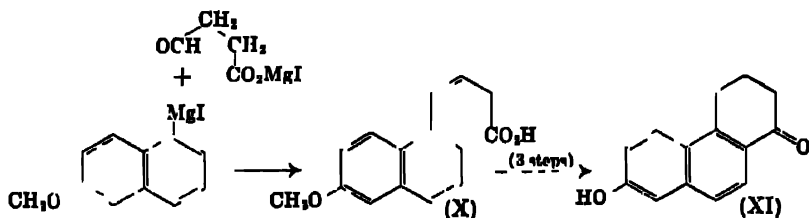
of sulfuric acid on the keto ester being cyclopentenophenanthrene. That the unsaturated ester VII (or a bond isomer) is the initial product can be inferred from the later work of Cook ⁷ who employed a similar direct cyclization and isolated a product analogous to VII.

These methods of ring closure are perhaps related also to that of the Bischler-Napieralski isoquinoline synthesis (page 33). Fieser and Hershberg ⁸ employed the von Auwers and Möller adaptation of the Bougault reaction for the preparation of 3,4-dihydrophenanthrene-1,2-dicarboxylic acid anhydride (IX). On condensing the keto ester VIII



with sulfuric acid at 80-90° the cyclic diester (analogous to V, above) is converted into the anhydride. In both this synthesis and that of Bardhan, the introduction of a hydroxyl group at the proper position should present no difficulties. 3,4-Dihydrophenanthrene-1,2-dicarboxylic acid anhydride (IX) can be converted into the corresponding aromatic anhydride in good yield by treatment with sulfur at 325°.

Butenandt and Schramm ⁹ prepared an interesting hydroxyketone of the phenanthrene series from the unsaturated acid (X) obtained as one product of the condensation of 6-methoxy-1-naphthyl magnesium iodide with the MgI-salt of succinic acid half-aldehyde. Hydrogenation of X,



⁷ Cook, Haslewood and (Mrs) A. M. Robinson, *J. Chem. Soc.*, 637 (1935)

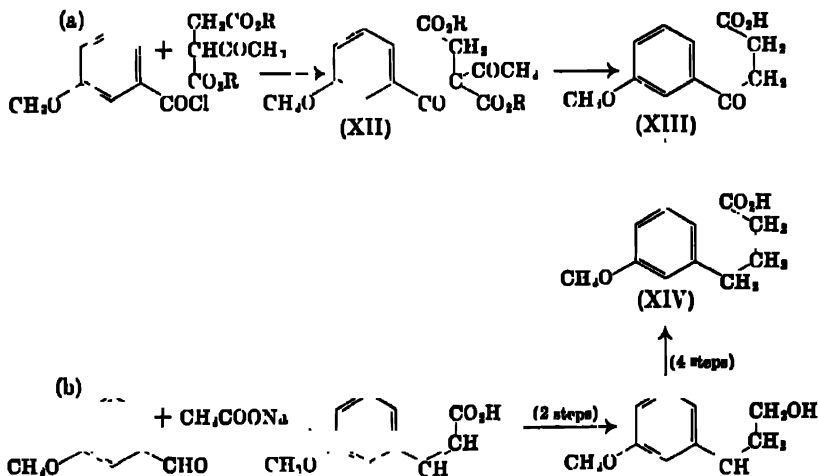
⁸ Fieser and Hershberg, *J. Am. Chem. Soc.*, 57, 1508, 1851 (1935)

⁹ Butenandt and Schramm, *Ber.*, 68, 2083, 2303 (1935)

cyclization of the free acid with stannic chloride, and demethylation gave the substance XI, which is the 7-hydroxy derivative of the ketone reported by Cook and Dodds⁹⁵ to be oestrogenically active.

The new substance was found inactive in the Allen-Doisy test up to 9 mg. when injected into castrated mice in 6 portions during 3 days. Administered by the same technique, 1-ketotetrahydrophenanthrene gave negative results in a total dosage of 70 mg., although 20-40 mg. of the ketone produced the oestrous response when the total amount was administered in a single injection, as in the experiments of the English investigators.

Still more elaborate are two syntheses reported from the laboratory of R. Robinson.¹⁰ These both require as starting material γ -*m*-methoxyphenylbutyric acid (XIV), for the preparation of which two methods have been employed. Thompson¹¹ (scheme a) used Mrs. G. M. Robinson's keto acid synthesis¹² for the preparation of β -*m*-methoxybenzoyl propi-



onic acid (XIII), which was obtained by condensing *m*-methoxybenzoyl chloride with the sodium derivative of ethyl acetyl succinate and hydrolyzing the product (XII). Reduction of XIII by the Clemmensen method gave the desired acid. The yields in both reactions were very poor, and for this reason Robinson chose the longer route (b), starting with *m*-methoxybenzaldehyde.

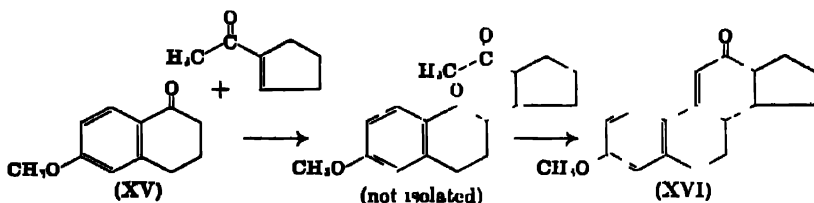
The first synthesis, described by Rapson and Robinson, employs a new general method of obtaining substituted cyclohexenones. 6-Methoxytetralone-1 (XV), prepared by cyclization of the acid XIV (above) with

¹⁰ Rapson and R. Robinson, *J. Chem. Soc.*, 1245 (1935); R. Robinson and Schlittler, *ibid.*, 1258 (1935). See also R. Robinson and J. Walker, *ibid.*, 1530 (1935); Rapson and R. Robinson, *ibid.*, 1533 (1935).

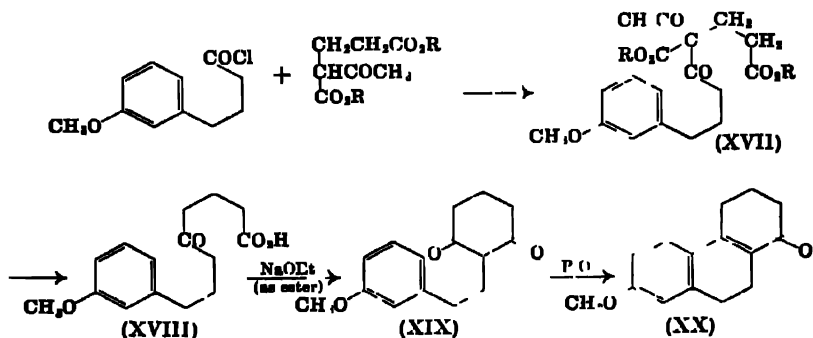
¹¹ H. W. Thompson, *ibid.*, 2814 (1932).

¹² G. M. Robinson, *ibid.*, 745 (1930).

sulfuric acid, was converted by means of sodamide into its sodium derivative and this was condensed with acetyl cyclopentene. The product of the Michael reaction was not isolated, for it underwent cyclization under the



conditions of the first condensation and gave the unsaturated ketone (XVI) in 55% yield. In the synthesis described by Robinson and Schlittler the phenanthrene system is constructed by effecting a double ring closure in a long-chain keto acid. The substance (XVIII) required for the purpose was prepared according to Mrs. G. M. Robinson's method by condensing γ -*m*-methoxyphenylbutyryl chloride with ethyl α -acetylglutarate (from acetoacetic ester and β -bromopropionic ester), and hydrolyzing the product (XVII). An intramolecular ester condensation gave the dihydroresorcinol derivative XIX, and the second cyclization was accomplished by treatment of XIX with phosphorus pentoxide. In this ring closure, which is essentially of the type of the Bougault reaction,



advantage is taken of the favorable orientation of the methoxyl group. The reaction product XX has shown no oestrogenic activity in small doses.

The synthetic phenanthrene derivatives III, IX, XI, XVI and XX are still far from the true hormone structures, but they all contain reactive groups which offer possibilities for further synthetic operations.¹²

¹² Other synthetic experiments undertaken with the view of obtaining similar hydrophenanthrene derivatives have been reported recently by Lehmann and Passche, *Ber.*, 68, 1148 (1935), Chuang, Ma and Tien, *ibid.*, 68, 1944 (1935), A. Cohen, *J. Chem. Soc.*, 420 (1935), A. Cohen and Cook, *ibid.*, 1870 (1935), A. Cohen, Cook and Hewitt, *ibid.*, 1833 (1935), Cook and Lawrence, *ibid.*, 1837 (1935), Chatterjee, *J. Indian Chem. Soc.*, 12, 418, 591 (1935), A. Cohen, *Nature*, 136, 869 (1935).

Clearly the progress made to date foreshadows rapid advances in the future.

THE MALE HORMONES

Isolation of Androsterone. Some reference was made above to the physiological experimentation leading to the recognition that there is present in the inner secretions of the testis a substance or group of substances exhibiting several different physiological properties of a hormonal character. The following functions are characteristic of the testicular hormone or hormones: the control of the development of the male genital organs (seminal vesicles, prostates, Cowper glands, vas deferens, and penis); the influence on the secretory activity of the accessory glands and on the character of the sperms; the development of the secondary sex characteristics. The most satisfactory methods for the detection and quantitative bio-assay of the hormone are those depending upon its influence on the development of the seminal vesicles of castrated male rodents (Loewe and Moore test) and upon the ability of the hormone to promote comb-growth in capons (roscumb test of Korb). The second method, which originated with Pézard¹⁴ and which was placed upon a quantitative basis by Gallagher and Koch,¹⁵ proved to be of particular value in the brilliant work of Butenandt and Tscherning¹⁶ which led to the first isolation of a male sex hormone in a pure, crystalline condition. According to the technique¹⁷ employed by these investigators, the capon unit (c. u.) is defined as the amount of substance which, when administered to each of three capons on two successive days, produces in the course of the third and fourth day an average increase of 20% in the area of the comb. The area is measured on a shadowgraph of the comb by means of a planimeter.

The isolation of male hormones was a matter of the utmost difficulty. The amount of materials present in the genital organs is extremely small and early attempts of several investigators to obtain pure material from testis tissue met with failure. Small amounts of a male hormone, however, occur in the blood and in the urine of healthy males, and the systematic investigation of the latter source by Butenandt and Tscherning eventually led to success. Using the comb growth test as a guide, and employing as the starting material a crude extract supplied by the Schering-Kahlbaum Company, these investigators achieved the first isolation of a crystalline hormone in 1931. Some idea of the difficulties involved can be gained from the fact that in the original work some 15,000 liters of urine yielded only 15 mg. of the hormone. Once the

¹⁴ Pézard, *Compt. rend.*, 153, 1027 (1911)

¹⁵ Gallagher and Koch, *J. Biol. Chem.*, 84, 495 (1929)

¹⁶ Butenandt and Tscherning, *Z. anorg. Chem.*, 44, 905 (1931)

¹⁷ *Idem*, *Z. physiol. Chem.*, 229, 167 (1934).

method of separation had been perfected, it was possible to obtain considerably higher yields. According to the bio-assay, 100 liters of male urine contains approximately 100 mg. of the hormone (500-600 c. u.) and about 25% of the substance present can be obtained in a pure condition.

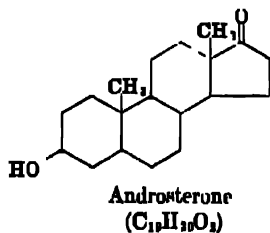
In the first step of the process¹⁸ the urine was acidified, concentrated, and extracted with chloroform. A large amount of inert material was removed from the chloroform solution by extraction with alkali, when the hormone was retained in the neutral fraction. Steam distillation removed an additional quantity of inactive material, and further enrichments were accomplished by subjecting the residue to alkaline hydrolysis and to hydrolysis with hydrochloric acid. The unhydrolyzed residue containing the hormone was then distributed between benzene and petroleum ether and extracted from the petroleum ether phase with 60% alcohol. At this point a highly active oil was obtained (1 c. u. = 1-1.4 mg.), but it was not improved by the further application of similar methods. In the course of the separations the male hormone had behaved so much like oestrone, except for the absence of acidic properties, that it seemed likely that the two substances are somewhat similar in structure. On the hypothesis that the male hormone is a hydroxyketone, the purified oil was treated with various reagents for the hydroxyl and for the carbonyl group in the search for a method of selective precipitation. By noting if there was a change in physiological activity it was possible to judge if a reaction had occurred. It soon was found that the active principle is ketonic in character, for with hydroxylamine a crystallizate was obtained which removed from the oil nearly all of its activity. The purified oxime yielded on hydrolysis a mixture of substances which could be separated by fractional sublimation in high vacuum and by crystallization. The principal component was a ketone melting at 178° and containing one capon unit in 150-200 γ (0.15-0.20 mg.) of material. On the basis of further chemical characterization, which indicated that the substance is a sterol-like ketone, Butenandt named the hormone androsterone (Gr., *andro-*, male).

Androsterone was found to be completely saturated, and the presence of an alcoholic hydroxyl group was established by the preparation of an acetate. The great difficulty of isolation imposed serious limitations on the work of characterization, and the combustion of the hormone presented a further difficulty. Reliable analyses of the acetate,¹⁹ however, pointed to the formula $C_{19}H_{28}O_2$ or $C_{19}H_{26}O_2$. Either formula would indicate a carbon skeleton containing four reduced rings, and the nature of both oxygen atoms was clear from the character of the derivatives. This information,

¹⁸ For a detailed account see Butenandt and Tschorning, Ref. 17, also, Tschorning, *Ergebnisse Physiol.*, 35, 801 (1932).

¹⁹ Butenandt, *Z. angew. Chem.*, 45, 655 (1932); *Naturwissenschaften*, 21, 49 (1933).

though meager, was remarkably suggestive (and accurate), for it pointed to a structural relationship to the sterols and a still closer relationship to oestrone, a hydroxyketone having a four-ring system and containing eighteen carbon atoms. On the basis of this analogy, Butenandt suggested what has proved to be the correct formula for androsterone at a



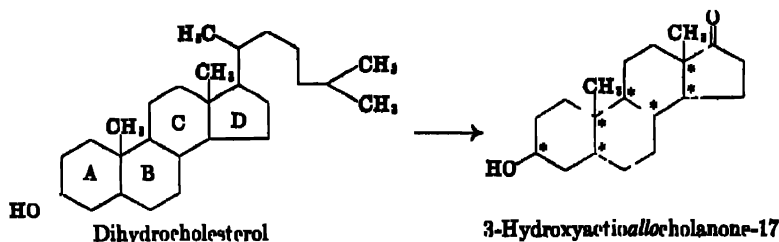
time (1932) when no more than 25 mg. of the hormone had been obtained in a pure condition!

According to this formula androsterone contains a methyl group at C_{10} , corresponding to that of the sterols. If this methyl group were removed and if ring A were dehydrogenated to an aromatic nucleus, the structure would be that of oestrone. Since, according to the analyses at the time available, the methyl group might be absent, it seemed possible that the hydrogenation of oestrone might yield androsterone. At Butenandt's suggestion, Schoeller, Schwenk and Hildebrandt²⁰ investigated the hydrogenation of oestrone, and they obtained an impure, non-crystalline product which was not identical with androsterone but which possessed the same type of physiological activity as the male sex hormone. In large doses the material gave a positive response in the capon test. This interesting observation in some measure indicated the probable validity of the type of formula suggested by Butenandt.

Preparation of Androsterone from Cholesterol. At the time when the problem had reached this highly suggestive but still uncertain stage, Ruzicka²¹ (1934) undertook an investigation of the question of structure by the preparative route. Assuming Butenandt's formula to be correct, it seemed altogether probable that androsterone arises in the organism as a degradation product of a reduced sterol or of lithocholic acid, and Ruzicka attempted to duplicate the degradation. The most readily available starting material was dihydrocholesterol. If this could be oxidized in such a way as to eliminate the side chain and introduce a ketonic oxygen atom in the position vacated, the resulting product would have the

²⁰ Schoeller, Schwenk and Hildebrandt, *Naturwissenschaften*, 21, 286 (1933); See also, Dürcherl and Voss, *ibid.*, 22, 315 (1934); Brit. patents, 421,681 (1934), 423,287 (1936).

²¹ Ruzicka, Goldberg and Brünnger, *Helv. Chim. Acta*, 17, 1388 (1934); Ruzicka, Goldberg, J. Meyer, Brünnger and Eichenberger, *ibid.*, 17, 1395 (1934); Ruzicka, Brünnger, Eichenberger and J. Meyer, *ibid.*, 17, 1407 (1934).

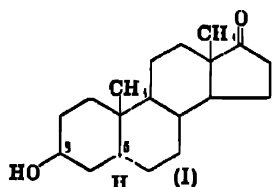


expected structure. According to the usual sterol nomenclature, this would be a hydroxyaetiocholanone. A hydroxyl-free ketone of this structure (but belonging to a different stereochemical series) had been isolated by Wieland, Schlichting and Jacobi in the course of the systematic degradation of the side chain of cholanic acid (page 146), but the aetiocholanone had been obtained only in very small amounts. Windaus had obtained no ketonic ring compounds in his oxidations of cholestane and coprostane, and it would seem that the presence of a hydroxyl group, even when protected by acetylation, would introduce the further complication of providing a vulnerable center for the opening of a ring.

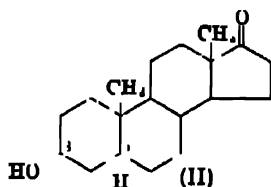
In spite of adverse indications from the literature, Ruzicka investigated the oxidation of dihydrocholesteryl acetate with chromic anhydride in boiling glacial acetic acid solution. Most of the material was either unattacked or converted into acidic substances, but there was a small neutral fraction which yielded a crystalline semicarbazone. On hydrolysis there was obtained a substance of the expected composition and chemical properties and showing a definite physiological activity in the comb growth test. The material was less active than androsterone and it did not correspond in melting point with this substance, but the general similarity was so great as to suggest that the two products are stereoisomers. There is ample opportunity for such isomerism in the hydroxyaetiocholanone structure. Corresponding to the seven asymmetric carbon atoms present, no less than 128 stereoisomeric forms are possible. Fortunately, however, the problem is much less complicated than the multiplicity of possible forms would indicate. The interconversions of various sterols and bile acids show clearly that rings C and D have the identical configuration in all of these natural products. The junction C/D is known to be *trans*, and X-ray data²² afford some indication of a *trans* linkage between rings B and C. All of the substances mentioned fall into one or the other of the two stereochemical series of which the basic hydrocarbons are cholestane (*allo*-series) and coprostane. If androsterone is formed in the organism from a sterol or bile acid it must belong to either the cholestane or the coprostane series, and if the hydroxyl group occupies

²² Bernal, *Chemistry and Industry*, 52, 11 (1933).

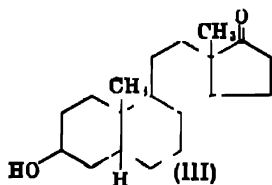
the usual C₁₃-position the configuration at this center of asymmetry must be that of dihydrocholesterol or of its epimer. In short it appeared probable that androsterone has the configuration of one of the four ketones, formulas I-IV, corresponding to dihydrocholesterol, coprosterol, and their



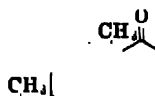
3-Hydroxy-5 α -cholestan-20-one-17
(from dihydrocholesterol)



3-Epihydroxy-5 α -cholestan-20-one-17
(from epi-dihydrocholesterol)



3-Hydroxy-5 β -cholestan-20-one-17
(from coprosterol)



3-Epihydroxy-5 β -cholestan-20-one-17
(from epi-coprosterol)

epimeric modifications. Having found it possible to obtain the first of these ketones by the oxidation of dihydrocholesteryl acetate, Ruzicka and his collaborators undertook the preparation of the other three isomers. The preparation of the reduced sterols required as starting materials has been discussed in the previous chapter (page 115), and it may be noted that at the time of the work under consideration epi-dihydrocholesterol and coprosterol had just become available through the discoveries of Vavon (1933) and of Grasshof (1934), respectively. A satisfactory method of preparing epicoprosterol was developed by Ruzicka.

The oxidation of the reduced sterols in the form of the acetates was in each case successful, and the four hydroxyketones were all obtained in a pure condition, if in small (and unspecified) yields. The ketone II, obtained as an oxidation product of epi-dihydrocholesterol, was found to be identical in every respect with the androsterone. The structure of the hormone, inferred by Butenandt from such observations as he was able to make with no more than 25 mg. of material available for experimentation, was in this way completely established!

Ruzicka's oxidation product corresponded very closely in physiological activity with the natural androsterone in parallel series of comb growth tests. The production of the active substance from an inactive starting material is a good proof of the pure hormonal character of the material

isolated from urine. A comparison of androsterone with the other stereoisomeric hydroxyketones is of interest in revealing the degree of specificity of the hormone. The figures given below represent the amounts of materials (in γ) corresponding to 1 capon unit (as determined by the technique of Tschopp):

Isoandrosterone (I):	500 γ = 1 capon unit
Androsterone (II):	60 γ = 1 capon unit
Compound III:	To 1000 γ inactive
Compound IV :	To 1000 γ inactive

From the lack of activity of the substances (III and IV) of the coprostane series it may be concluded that the configuration of the ring system is of more importance than the configuration of the carbon atom carrying the hydroxyl group, although an inversion at this part of the molecule results in a drop in physiological activity to about one-seventh the original value.²³ The specific action of androsterone in comparison even with its stereoisomerides may be contrasted with the lack of specificity of the oestrus-producing hormones. It is worthy of note that the male hormone belongs to the cholestane (*allo*) series, while pregnanediol corresponds in configuration to coprostane.

It was surprising to find that androsterone is an alcohol of the *epi*-type, for such substances previously had not been known to occur in nature. Certain sterols and bile acids, however, had not been characterized with respect to the stereochemical arrangement of the hydroxyl group, and in some cases even the position of the alcoholic group was unknown. Ruzicka's method of oxidation provided a convenient means of deciding these points and it was soon found that stigmasterol,²⁴ ergosterol,²⁴ cinchol,²⁵ and sitosterol²⁶ are 3-hydroxy compounds of the cholesterol (normal) type, but that lithocholic acid²⁷ is an *epi*-compound, for it yields the hydroxyketone (IV) obtained from *epicoprosterol*.

Ruzicka's method for the artificial preparation of androsterone²⁸ has made the hormone much more readily available for experimentation and for clinical use, but it is an extravagantly wasteful process which finds practical application only because the starting material is available at a low price. From 3 kg. of cholesterol, Callow and Deanesly²⁹ obtained in all 1 g. of androsterone, although under the most favorable conditions

²³ This observation was confirmed by Butenandt and Cöhler, *Z. physiol. Chem.*, **234**, 219 (1935), who prepared 3-hydroxyandrosthenolone-17 (isoandrosterone) by a stepwise degradation of stigmasterol. See also Wallin and Fernholm, *J. Am. Chem. Soc.*, **57**, 1511 (1935); Dirscherl, *Z. physiol. Chem.*, **235**, 1 (1935).

²⁴ Fernholm and Chakravorty, *Ber.*, **67**, 2021 (1934).

²⁵ Dirscherl, *Z. physiol. Chem.*, **235**, 1 (1935).

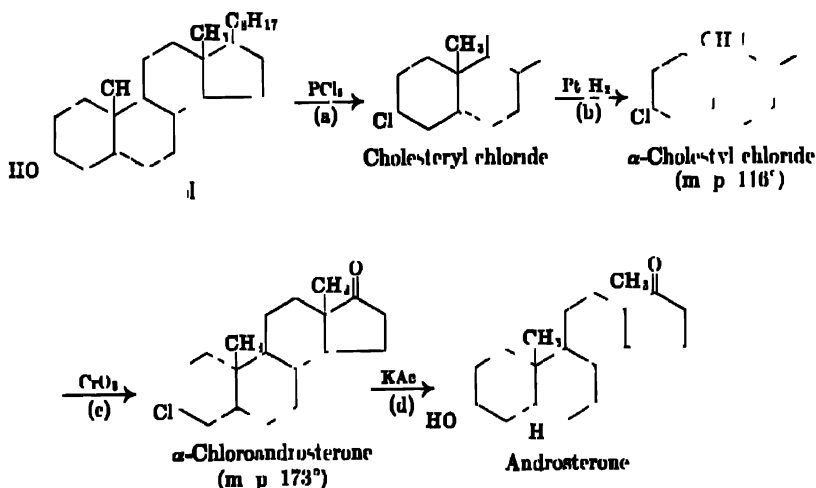
²⁶ Ruzicka and Eisenberger, *Helv. Chim. Acta*, **18**, 430 (1935).

²⁷ Ruzicka and Goldberg, *ibid.*, **18**, 668 (1935).

²⁸ French patent 779,132 (1935).

²⁹ Callow and Deanesly, *Biochem. J.*, **29**, 1424 (1935).

the overall yield rose to 0.2%. To obtain 1 g. of the natural hormone it is necessary to use about 4,000 liters of male urine. Callow and Deaneley used Girard's reagent (page 197) for separating the desired ketone from the mixture of oxidation products, and they made improvements in other steps. To recapitulate, the complete process involves the following transformations: cholesterol \longrightarrow dihydrocholesterol \longrightarrow cholestanone \longrightarrow *epidi*hydrocholesterol \longrightarrow acetate \longrightarrow androsterone acetate (semi-carbazone) \longrightarrow androsterone. According to Marker³⁰ a more convenient process consists in the use of the chloro derivative in place of the acetate, for only one hydrogenation is required and the materials are more easily handled in large lots. The steps are shown in the formulas.



Since androsterone has the opposite configuration at C_1 from cholesterol, a Walden inversion must take place at one of the steps but it is not known whether this occurs in the replacement of hydroxyl by chlorine (a) or in the reverse change (d). Ruzicka³¹ obtained the same α -chloroandrosterone (m.p. 173°) by the oxidation of α -cholestyl chloride prepared from *epidi*hydrocholesterol and phosphorus pentachloride, and the substance proved to be a stereoisomer of the chloroketone (m.p. 128°) obtained by the sequence: dihydrocholesterol $\xrightarrow{\text{PCl}_5}$ β -cholestyl chloride (m.p. 105°) $\xrightarrow{\text{CrO}_3}$ β -chloroandrosterone (m.p. 128°).³² The stereochemistry of the replacement reactions still awaits elucidation.

The preparation of androsterone by the systematic degradation of the side chain of a bile acid by the method of Wieland, Schlichting and Jacobi

³⁰ Marker, *J. Am. Chem. Soc.*, **57**, 1755 (1935); Marker, Whitmore and O. Kamm, *ibid.*, **57**, 2358 (1935).

³¹ Ruzicka, Wirs and J. Meyer, *Helv. Chim. Acta*, **15**, 998 (1932).

³² Ruzicka, Goldberg and Brüniger, *ibid.*, **17**, 1389 (1934).

has been investigated by Dalmer, v. Werder, Honigmann and Heyns.³³ According to Ruzicka's observations, the natural bile acids have the desired *epi*-configuration at C₁₃, but they belong to the coprostane rather than the cholestane series. 3-Hydroxyallocholanolic acid proved to be a suitable starting material. It was found that the ester of this acid can be degraded with little modification in the standard Grignard procedure, the acetylated hydroxyl group easily surviving the oxidations. In the final step the diphenyl carbinol from 3-hydroxyaetionallocholanolic acid was dehydrated and submitted to ozonization. Pure androsterone was obtained. These investigators also prepared 3-hydroxynorallocholanolic acid, one of the intermediates in the above degradation, by the oxidation of the acetates of *epistigmastanol* and *epistostanol*, which were obtained for the purpose by the hydrogenation of the corresponding saturated ketones in an acid medium, following Vavon's procedure. The degradation of lithocholic acid to nor- and bisnor-lithocholic acid by the oxidation of the Grignard products has been described by Reindel and Niederlander.³⁴ By a process parallel to that of Ruzicka, Dirscherl³⁵ has obtained androsterone from cinchol, *epidi*hydrocinchol being prepared as above and oxidized as the acetate.

Characterization of Androsterone. The preparation of androsterone by the degradation of a sterol, or by what may be termed a pseudo-synthesis, settled in a remarkably short space of time the problem of the structure of one of the most difficultly accessible natural products. Subsequent events have shown, however, that at the time of the announcement of Ruzicka's important work the problem had been nearly completely solved by the degradative route in the investigations of Butenandt and his collaborators. The systematic working¹⁷ of the crude extract from a large quantity of male urine over a period of three years had made available by the middle of 1934 a total of 1.5 g of pure androsterone, and with the aid of suitable methods of microtechnique this sufficed for a rather elaborate characterization of the hormone. Some of the properties, for the most part as recorded by Butenandt and Tschern-

PROPERTIES OF ANDROSTERONE

Formula	M p, corr.	[α] _D	Physiol activity, r u	Acetate m p	Oxime m p	Semi- carbasone, m p.
C ₂₁ H ₃₀ O	182-183°	+94.5°	150-200 γ	161°	216°	276°

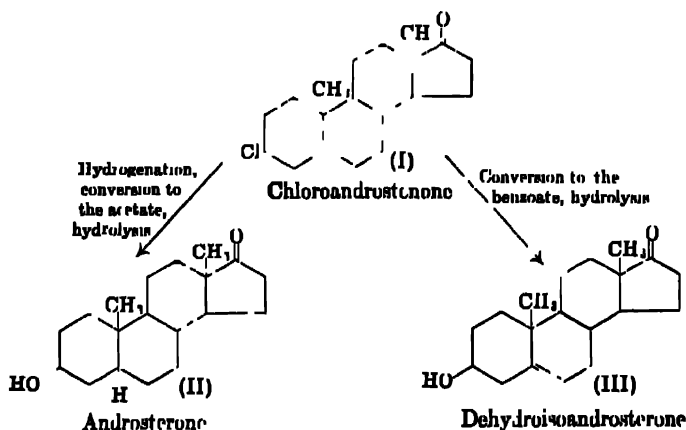
³³ Dalmer, v. Werder, Honigmann and Heyns, *Ber.*, 68, 1814 (1935)

³⁴ Reindel and Niederlander, *ibid.*, 68, 1960 (1935)

³⁵ Dirscherl, *Z. physiol. Chem.*, 237, 52, 268 (1935)

ing,³⁶ are reproduced in the table. The hydroxyketone yielded androstenedione on oxidation, and on reduction of the diketone by the Clemmensen method the saturated hydrocarbon androstane (m.p. 49-50°) was obtained. This parent hydrocarbon was found to be different from the aetiocholane (m.p. 78-80°) obtained by the reduction of Wieland's aetiocholane-17, and it is clear from Ruzicka's work that androstane belongs to a different (the *allo*) stereochemical series.

Dehydroisoandrosterone, a Second Hormone from Urine. The most enlightening observations centered around the isolation by Butenandt and Dannenbaum³⁷ of two companion substances in the hormone extract. The more abundant of these substances was a physiologically inactive, unsaturated chloroketone (m.p. 157°) which evidently had been formed in the course of the isolation by the action of hydrogen chloride on an unsaturated hydroxyketone during the acid hydrolysis step of the enrichment. It was possible to obtain such a ketone by the interaction of the chloro compound with potassium benzoate, followed by hydrolysis of the resulting benzoyl derivative. The unsaturated hydroxyketone itself subsequently was isolated from urine in small amounts,³⁸ and a relationship to androsterone was established by the transformation of the unsaturated chloroketone into this substance by the hydrogenation, conversion to the acetate, and hydrolysis. From more recent evidence (see below) it is known that the unsaturated hydroxyketone has the structure and the configuration at C₉ represented in formula III, which indicates the relationship of this substance and of the chloro compound (I) to



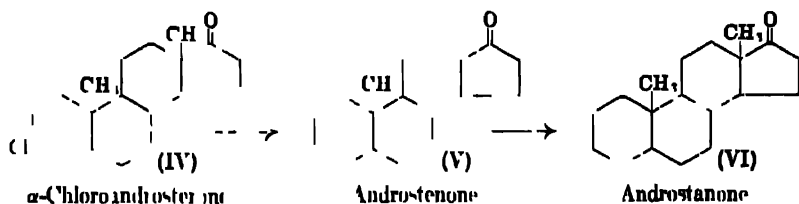
³⁶ Butenandt and Tscherning, *Z. physiol. Chem.*, **229**, 185 (1934).

³⁷ Butenandt and Dannenbaum, *ibid.*, **229**, 192 (1934). See also Butenandt, Dannenbaum, Hanisch and Kudasus, *ibid.*, **237**, 57 (1935).

³⁸ Butenandt and Tscherning, *ibid.*, **229**, 197 (1934).

androsterone (II). While androsterone is an *epi*-compound, III is of the normal type of cholesterol and an inversion occurs in the transformation of the unsaturated chloroketone (I) into either II or III. Butenandt named the unsaturated hydroxyketone "dehydroandrosterone" before this point had been established, but in view of the epimeric relationship the name requires modification. The hormone is a dehydro derivative of isoandrosterone (3-hydroxy-actioallocholanone-17) and it is appropriately called dehydroisoandrosterone.³⁹ The compound has a characteristic physiological action similar to that of androsterone, but it is less potent.

The observation in Butenandt's laboratory that chloroandrostenone (I) gives a characteristic Liebermann-Burchard test afforded a preliminary, if unreliable⁴⁰ indication of the sterol-like nature of the substances of the androsterone group. The saturated chloroketone IV (m.p. 173°), identical with the compound prepared later by Ruzicka⁴¹ and by Marker,³⁹ was converted by Butenandt and Dannenbaum³⁷ under special conditions into the unsaturated ketone V. This afforded on hydrogenation the saturated, hydroxyl-free ketone androstanone (VI). The sub-



stance was found to be different from Wieland's actiocholanone-17, and at the time at which the work was carried out the ketone was unknown. It was not long, however, before the preparation from sterols of this important degradation product was accomplished independently by Fernholz and Chakravorty⁴¹ and by Ruzicka, Goldberg and Wirz.⁴⁰ The method consisted in the oxidation of cholestane with chromic anhydride, and the yield was only a fraction of one per cent. The product, which from the method of preparation must have the structure and configuration of actioallocholanone-17, was identical with androstanone. The degradative work, considered independently of the pseudo-synthesis of the hormone, establishes the structure and the configuration of androsterone in every detail save the exact location and spatial arrangement of the secondary hydroxyl group.

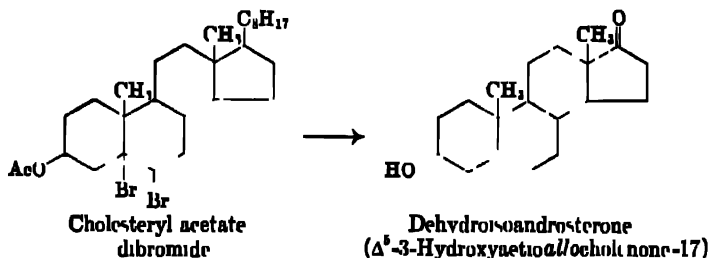
The hormone dehydroisoandrosterone is particularly interesting because it is intermediate in degree of unsaturation between androsterone

³⁹ Ruzicka (Ref. 40) uses the term "trans-dehydroandrosterone," the prefix referring to the (arbitrarily assumed) relationship between the hydroxyl group and the hydrogen atom at C-1 of the hydrogenation product.

⁴⁰ Ruzicka, Goldberg and Wirz, *Helv. Chim. Acta*, 18, 61 (1935).

⁴¹ Fernholz and Chakravorty, *Ber.*, 68, 353 (1935).

and oestrone, and it may be a natural precursor of substances of both groups. The isolation of dehydroisoandrosterone attracted considerable interest and the preparation of the hormone from sterols was soon reported from five different laboratories. Ruzicka and Wettstein,⁴² and Wallis and Fernholz⁴³ used the identical method, protecting the hydroxyl group of cholesterol by acetylation and the double bond by the addition of bromine, and eliminating the side chain by oxidation. After debromina-



tion the ketone was isolated as the acetate semicarbazone in about 1% yield, and the unsaturated hydroxyketone obtained on hydrolysis proved to be identical with Butenandt's dehydroisoandrosterone. This clearly establishes the position of the double bond and the configuration at C₅. At the Amsterdam laboratory, David⁴⁴ reported finding as much of the unsaturated hormone in urine as androsterone, and Oppenauer⁴⁵ prepared a substance identical with the natural material from sitosterol of soya beans by a process similar to that described above. Schoeller, Serini and Gehrke⁴⁶ noted that the hormone is precipitated by digitonin (normal type) and stated that a preparation had been achieved, but they reported no details. Butenandt⁴⁷ later reported the preparation of dehydroisoandrosterone from stigmasterol, as well as from cholesterol (yield, 2.8%), by the above method.

Physiological Activity of Derivatives of the Hormones. Although the difficulty of obtaining large quantities of androsterone and dehydroisoandrosterone either from natural sources or by the degradation of cholesterol is a handicap to the biological experimentation with the hormones, a fairly extensive investigation of the physiological activity of androsterone derivatives has been reported from Butenandt's laboratory,¹⁷ and the observations have been confirmed and extended by Ruzicka,⁴⁸ using a slightly different test method (Tschopp technique).

⁴² Ruzicka and Wettstein, *Helv. Chim. Acta*, **16**, 986 (1935).

⁴³ Wallis and Fernholz, *J. Am. Chem. Soc.*, **57**, 1376, 1504 (1935).

⁴⁴ David and Freud, *Acta Brevia Neerland.*, **5**, 31 (1935).

⁴⁵ Oppenauer, *Nature*, **135**, 1030 (1935).

⁴⁶ Schoeller, Serini and Gehrke, *Naturwissenschaften*, **23**, 337 (1936).

⁴⁷ Butenandt, Dannenbaum, Hanisch and Kudasus, *Z. physiol. Chem.*, **237**, 57 (1935).

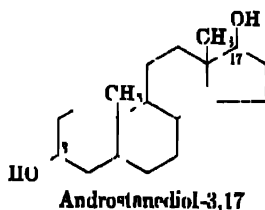
⁴⁸ Ruzicka, Goldberg and J. Meyer, *Helv. Chim. Acta*, **18**, 210 (1935).

A comparison of the physiological activity of these derivatives and transformation products as determined by Butenandt's technique is given in the accompanying table.

COMB GROWTH ACTIVITY OF ANDROSTERONE DERIVATIVES

	M. p.	Capon unit equivalent
Androsterone	182°	150-200γ
Androsterone acetate	161°	150-170γ
Androsterone oxime	216°	1000γ
Androstenediol-3,17	221°	45-50γ
Androstenediol-3,17 diacetate	180°	60-70γ
Androstenedione-3,17	126°	300γ
Androstanone-17	122°	2000-3000γ
Dihydroandrosterone (two polymorphic forms)	148°, 138°	600γ

The acetylation of androsterone or of other alcohols of the series does not alter the effective dose but the acetates exhibit a markedly protracted action. It is not known whether this is because time is required for hydrolysis in the organism to the alcohols, or because the process of resorption is slower in the case of the acetates. The hormonal activity is retained to some extent in the oxime and in the mono- and di-ketones derived from the parent hydrocarbon androstane. The most striking observation is that the dihydro compound resulting from the reduction



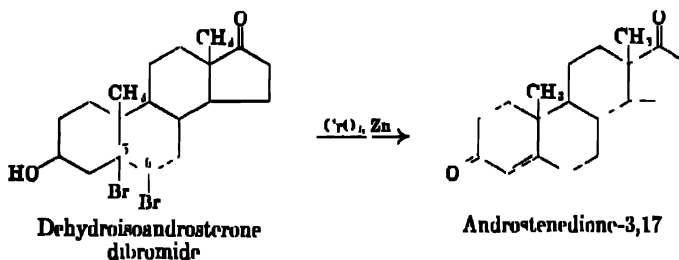
of the carbonyl group of the hormone is three times as active as androsterone. The diol was prepared by Butenandt⁴⁹ by reduction with sodium and propyl alcohol and by Ruzicka⁴⁹ by the hydrogenation of androsterone in an acid solution (m. p. 223°, corr.). It will be recalled that the physiological activity of oestrone is also increased to a marked degree by the reduction of the ketonic group. By the action of methyl magnesium iodide on androsterone, Ruzicka⁵⁰ prepared 17-methylandrosterone-3,17, and this diol likewise was found to be more active than the parent substance. According to the Tschopp test the capon unit of the substance was 25γ in comparison with 60γ for androsterone. It is inter-

⁴⁹ Butenandt and Tscherning, *Verhandl. deutsch. Ges. Medizin.*, 46 Kongr., 203 (1934); *Z. physiol. Chem.*, 234, 224 (1935).

⁵⁰ Ruzicka, Goldberg and J. Meyer, *Helv. Chim. Acta*, 18, 994 (1935).

esting that the male hormone action is not limited to compounds having the androstane carbon-skeleton.

Of particular importance is the unsaturated diketone, androstenedione (m. p. 173°), prepared from dehydroisoandrosterone by Ruzicka and Wettstein,⁴² by Wallis and Fernholz,⁵¹ and by Butenandt and Kudasus⁵² by the oxidation of the dibromide and debromination. The double bond,



originally at C_5-C_{10} , migrates to a position of conjugation in the course of the latter reaction. In the Tschopp test, androstenedione is nearly as active (capon unit, 100γ) as androsterone (60γ). The substance resembles progesterone in structure, but it has no corpus luteum activity. There is a close structural relationship to oestrone, for the elimination of the elements of methane from the dione would give a substance having the structure of this hormone. While androsterone has no oestrogenic activity,⁵³ Butenandt^{52, 54} found that on injecting androstenedione into immature female rats and mice the substance produced a characteristic oestrous response. (With spayed animals the results were negative.) Dehydroisoandrosterone behaved in the same manner, and possibly both of these substances of male hormone activity are capable of being transformed into the female hormone in the ovaries. Since androstenedione, in addition, doubtless can yield both androsterone and dehydroisoandrosterone on hydrogenation, it may be the physiological precursor of substances of both the oestrone and the androsterone group. In addition to these important attributes, androstenedione bears a close relationship to a highly active male hormone of the testes, which will be described separately.

Testosterone. In the foregoing investigations of the hormones obtained from urinary extracts, bio-assays of preparations and derivatives were made for the most part by the convenient comb growth method. There are some differences in the capon unit as defined by the procedures of different investigators (Butenandt, Tschopp, Fussgänger,⁵⁵ Ogata⁵⁶)

⁵¹ Wallis and Fernholz, *J. Am. Chem. Soc.*, **57**, 1511 (1935).

⁵² Butenandt and Kudasus, *Z. physiol. Chem.*, **237**, 75 (1935).

⁵³ Warren, *Nature*, **135**, 234 (1935).

⁵⁴ Butenandt and Hanisch, *Ber.*, **68**, 1859 (1935).

⁵⁵ Fussgänger, *Med. u. chem. Abhandl. med.-chem. Forschungsinstitut*, **1**-**U**. Farbenind., **213** (1933).

⁵⁶ Ogata, Hirano and Tanaka, *J. Pharm. Soc. Japan*, **54**, 49 (1934).

but there is good agreement in the relative order of activity found for a series of compounds by these different techniques. Following the isolation of androsterone and dehydroisoandrosterone, and after methods had been developed for the preparation of the hormones from cholesterol, it became important to investigate the other biological properties of the pure substances. That androsterone influences the growth of the seminal vesicles was reported from Butenandt's laboratory.⁵⁷ Tschopp (Ruzicka, 1934) found that the prostate, seminal vesicles, and penis of castrated rats became enlarged after the injection of androsterone, and the observations were extended by Korenchevsky.⁵⁸ The effects were qualitatively similar to those known from an abundance of earlier observations to be produced by testicular extracts, and from the rough correspondence in the physiological properties it appeared possible for a time that the male hormones excreted in the urine are identical with those present in the testes. It was recognized that this important point might be decided either by the isolation of a pure male hormone from the testes or by direct, quantitative comparisons of the various physiological actions of urinary and testicular hormone extracts. Both lines of inquiry were actively investigated, and it soon became apparent that there are distinct differences in both the chemical and the biological properties of the active materials from the two sources.⁵⁹ Of particular significance were the results of a comparison made by Laqueur and his associates (1935) at Amsterdam of the effect of administering to castrated rats equal amounts, in terms of capon units, of urinary and of testicular male hormone extracts. The animals showed extraordinary differences in the size of the seminal vesicles. The average weights in the case of the controls, the animals receiving urinary extracts, and those receiving testicular extracts were in the ratios: 1:14:67. The material from testes has about five times the activity per capon unit in promoting the growth of the seminal vesicles as androsterone. The marked divergence in the relationship between the rat tests and the capon-potency clearly pointed to the presence in the testes of a substance other than androsterone.

Further evidence in the same direction was supplied by the observation of Gallagher and Koch (1935) that highly active testicular extracts show a decided decrease in activity when boiled with alkali. A similar instability is not characteristic of the urinary hormones, androsterone and dehydroisoandrosterone, but is exhibited by the α,β -unsaturated

⁵⁷ Tscherning, *Ergebnisse Physiol.*, 35, 312 (1933).

⁵⁸ Korenchevsky, *Nature*, 135, 484 (1935); Korenchevsky and Dennison, *Biochem. J.*, 29, 1720, 2122 (1935); Korenchevsky, Dennison and S. L. Simpson, *ibid.*, 29, 2131, 2334 (1935).

⁵⁹ Laqueur and Münch, *Ber. ges. Physiol.*, 61, 3 (1931); Dingemans, Freud and Laqueur, *Nature*, 135, 184 (1935); David and Freud, *Acta Bressa Nederland.*, 5, 13 (1935); Gallagher and Koch, *Endocrinology*, 18, 107 (1934); *J. Biol. Chem.*, 104, 611 (1934); Matsumaki, *Japan J. Med. Sci.*, 7, No. 1 (1934); Ogata and Hirano, *J. Pharm. Soc. Japan*, 54, 11 (1934); Callow and Dennison, *Biochem. J.*, 29, 1424 (1935); *Lancet*, II, 77 (1935).

ketone progesterone, from the corpus luteum. This relationship was commented on by Ruzicka and Wettstein and by Wallis and Fernholz in their work on the preparation of androstenedione-3,17, and it was pointed out that this alkali-unstable, α,β -unsaturated ketone may be closely related to the true testicular hormone.

The problem of the identity of the hormone was solved with surprising promptitude. In June, 1935, David, Dingemans, Freud and Laqueur⁶⁰ reported the isolation in a pure condition of a testicular hormone (m. p. 154°) to which they gave the name testosterone. About 10 mg. of the hormone was obtained from 100 kg. of testis tissue.⁶¹ The preparation from an androsterone derivative of a substance of known structure having chemical and physiological properties identical with those of testosterone was reported by Butenandt^{54, 62} in September, and shortly afterwards by Ruzicka.⁶³ It had been observed by both investigators that among the known compounds of the androsterone group the high potency in the rat test as compared with the capon test, characteristic of the testicular extracts (and of testosterone), was exhibited only by androstenedione-3,17.⁶⁴ Furthermore, this unsaturated dione, like testosterone, is unstable to alkalis, particularly when in an impure condition. While a close relationship between the two substances was apparent from these observations, the complete identity of the materials was subject to question even before the isolation of the natural hormone had been reported. Androstenedione-3,17 is somewhat less potent in the capon test than androsterone, whereas there were definite indications that the testicular hormone is considerably more powerful in this respect, as well as in the rat test, than androsterone. Laqueur indeed found that pure testosterone is about ten times as powerful as androsterone in promoting comb growth, the capon unit being contained in about 10% of the material. The activity in the rat test per capon unit is about seven times that of androsterone.

Since previous experience had shown that the physiological potency of androsterone is greatly enhanced by the reduction of the C₁₇ carbonyl group, both Butenandt and Ruzicka undertook the preparation of the corresponding derivative of androstenedione-3,17. Both investigators employed the same method. Dehydroisoandrosterone (I) was reduced with sodium and propyl alcohol to the diol II (m. p. 177-178°, slight activity in the capon test), and this was converted into the diacetate. Utilizing the greater reactivity of the 3-substituent, the diacetate was partially saponified to the monoacetate III. This was oxidized as the monoacetate

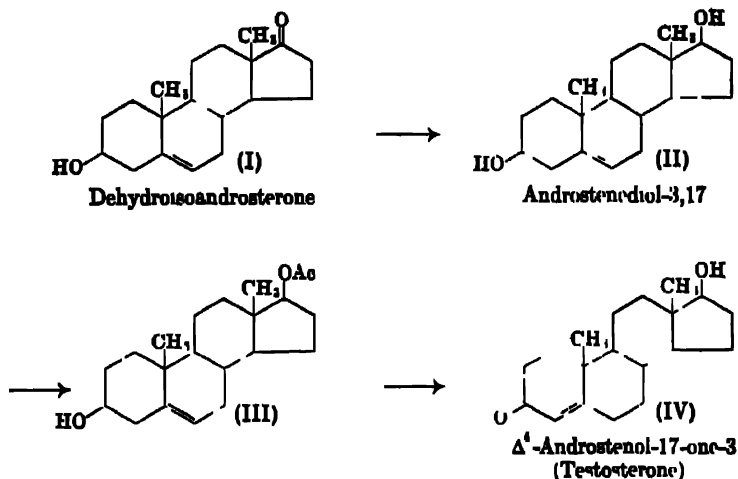
⁶⁰ David, Dingemans, Freud and Laqueur, *Z. physiol. Chem.*, **233**, 218 (1935).

⁶¹ David, *Acta Brera Neerland.*, **5**, 85, 108 (1935).

⁶² Butenandt and Hanisch, *Z. physiol. Chem.*, **237**, 89 (1935).

⁶³ Ruzicka, *J. Am. Chem. Soc.*, **57**, 2011 (1935); Wettstein, *Schweiz. med. Wochenschr.*, **61** (1935); Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 1264 (1935).

⁶⁴ See Tschopp, *Nature*, **136**, 258 (1935).



dibromide and the product on debromination and hydrolysis gave the unsaturated hydroxyketone IV (isomeric with I). The properties of Δ^4 -androstenol-17-one-3 (IV) as determined by both investigators agree well with those reported⁶¹ for testosterone. Furthermore, testosterone has an absorption spectrum characteristic of an α,β -unsaturated ketone

PROPERTIES OF TESTOSTERONE

Formula	M p, cori	$[\alpha]_D$	Physiol activity, capon unit (Tschopp)	Acetate m. p. corr	Osime, m p cori	Benzoate, m p corr
$C_{19}H_{28}O$	154-154.5°	+ 109'	$\approx 10\gamma$	140-141°	222-223°	194-196°

(maximum at 238 $m\mu$) and it yields androstenedione-3,17 on oxidation. Finally, mixed melting point determinations established completely the identity of the natural hormone with the synthetically prepared substance. Later Ruzicka⁶⁵ improved the yield in the preparation by substituting for the 3,17-diacetate of II the 3-acetate-17-benzoate of this diol, prepared by reducing acetyl dehydroisoandrosterone and benzoylating the product. With the mixed ester there was a greater differentiation in the ease of hydrolysis of the two groups than in the above case.

The isolation and identification of testosterone represents a highly important advance in the hormone field. From present indications it appears likely that testosterone is the characteristic, if not the only, male hormone of the testes. Clearly the hormones excreted in male and female urine are not the original hormones of the testes and ovaries, and they are

⁶⁵ Ruzicka, Wettstein and Kagi, *Helv Chim Acta*, 18, 1478 (1935).

less active than these substances. Androsterone and oestrone appear to be either transformation products of the true hormones of the genital glands or else companion substances. The occurrence of these materials in certain urines in relatively large amounts was a fortunate circumstance, for without this comparatively ready source of physiologically active substances for chemical investigation the characterization of the true hormones probably would have been long delayed.

It is quite possible that urinary and testicular extracts contain still other male hormones, and indeed Ogata and Hirano⁶⁶ have reported the isolation from 80 kg. of boar testes of 2 mg. of an alkali-stable substance melting at 129-130° and possessing a high degree of physiological activity. Ruzicka and Wettstein⁶⁸ noted that in melting point and in the action on the sexual organs of immature rats the substance resembles androstenedione (m. p. 129° or 132°), and they suggest that the two substances may be identical.

A preliminary characterization of compounds related to testosterone has been reported by Butenandt.⁶⁷ The saturation of the 4,5-double bond, accomplished by partial hydrogenation in neutral solution, resulted in a decrease in the comb growth activity, although the resulting androstanol-17-one-3 (*allo-series*) is considerably more potent than androsterone. Δ^4 -Androstenediol-3,17, prepared by the reduction of testosterone at the C₃-carbonyl group by means of aluminum isopropylate, was found to have little if any activity in the caapon test. Ruzicka has prepared and tested additional derivatives and transformation products of dehydroisoandrosterone⁶⁸ and of testosterone.⁶⁹ Δ^4 -Dehydroisoandrosterone was found to be more active than the Δ^5 -isomer found in urine. In comparing testosterone with various methyl carbinols, such as 17-methyltestosterone and 17-methylandrostanone-3-ol-17, it was surprisingly found that the latter saturated hydroxyketone approaches the testicular hormone in activity in both the caapon and the rat test. Testosterone, however, is the most potent substance of male hormone activity yet discovered.

THE CORPUS LUTEUM HORMONE, PROGESTERONE

In the human ovary, following the ripening and rupture of the follicle, there is formed a tissue which, on account of the abundance of yellow carotene present, is called the corpus luteum or yellow body. Physiological studies of the past thirty years⁷⁰ have shown that the main functions of the corpus luteum are connected with the preparation for and main-

⁶⁶ Ogata and Hirano, *J. Pharm. Soc. Japan*, **54**, 199 (1934)

⁶⁷ Butenandt, Tscherning and Hanisch, *Ber.*, **68**, 2097 (1935)

⁶⁸ Ruzicka, W. Fischer and J. Mayer, *Helv. Chim. Acta*, **18**, 1483 (1935)

⁶⁹ Ruzicka, Goldberg and Rosenberg, *ibid.*, **18**, 1487 (1935)

⁷⁰ See F. L. Hissaw, "Physiology of the Corpus Luteum," pp 400-543 in E. Allen's "Sex and Internal Secretions" (1932).

tenance of pregnancy. The uterine mucosa, which grows to a certain stage under the influence of the follicular hormone, proliferates further under the stimulation of secretions from the corpus luteum and so is prepared for the reception of the fertilized ovum. If no fertilization occurs, the corpus luteum degenerates after about two weeks and the excess mucosa is carried away in the menstrual flow. A new one ordinarily appears in the course of the next menstrual cycle. In the event of fertilization, the corpus luteum undergoes no regression but performs the following functions: it (1) suppresses ovulation, (2) maintains a condition in the uterus essential to the development of the embryo, (3) inhibits uterine motility, and (4) in conjunction with oestrin it induces mammary gland development.

That the corpus luteum is a gland with inner secretions, was suggested by the work of Fraenkel⁷¹ (1903), who showed that with rabbits the removal of the corpora lutea shortly after ovulation terminates pregnancy, or prevents the attachment of the ovum to the uterus. In 1928 the American investigators G. W. Corner and W. M. Allen⁷² made the important observation that, after the progestational changes have been prevented by the extirpation of the early corpus luteum, activity can be restored by the use of extracts of corpora lutea. Rabbits castrated shortly after mating and then given subcutaneous injections of such extracts develop within a few days a progestational condition similar to that during early pregnancy. Implantation of the fertilized egg occurs and pregnancy can be maintained to the point of normal birth. These experiments proved conclusively that the internal secretions of the corpus luteum contain a hormone which controls pregnancy.

The fundamental work of Corner and Allen not only furnished the basis for the chemical investigations of the corpus luteum hormone, but also provided a convenient test method. Adult female rabbits required for the test are castrated at the proper stage of follicle ripening, as determined by mating. The administration of a hormone extract produces after the fifth day a progestational proliferation of the mucosa which is easily recognized by histological examination. The test of Corner and Allen affords a reliable means for the quantitative assay of hormone preparations. Following other observations of these workers, Clauberg⁷³ developed a modified test⁷⁴ which dispenses with castration and with histological controls. In place of the oestrous type of uterine mucosa of the adult animal, a similar endometrium is produced artificially in the

⁷¹ Fraenkel, *Arch. Gynaekol.*, 68, 438 (1903)

⁷² Corner and W. M. Allen, *Am. J. Physiol.*, 86, 74 (1928); 88, 326 (1929), W. M. Allen and Corner, *ibid.*, 88, 840 (1929)

⁷³ C. Clauberg, "Die weibliche Sexualhormone," Berlin (1933).

⁷⁴ Rutenandt, U. Wentphal and Hohlweg, *Z. physiol. Chem.*, 227, 84 (1934)

infantile rabbit by the administration of follicular hormone for six to eight days. This serves as an indicator of the progestational activity of a preparation which is to be tested and the characteristic changes can be observed after the substance has been injected over a period of five days. Both methods are remarkably accurate, but care must be taken to exclude from the preparation submitted to test appreciable amounts of oestrin, for the follicular hormone has an antagonistic action when administered along with the corpus luteum hormone.

Isolation and Properties. Following the investigations of Corner and Allen, the younger of these workers, W. M. Allen, developed methods for the extraction and concentration of hormone preparations which were of the greatest value in advancing the field.⁷⁵ By 1932 physiologically active, if admittedly very crude, crystallizates had been secured from the corpus luteum by Allen⁷⁶ at Rochester, and by the research groups of F. L. Hisaw⁷⁷ at Wisconsin and of H. K. Slotta⁷⁸ at Breslau. The preparations were active in doses of as little as 3 mg., but the degree of purity was not sufficient to permit a chemical characterization.

The isolation of the pure hormone was beset with many difficulties. The starting material was expensive and it contained only traces of the active principle. The hormone, particularly in an impure condition, was found to be very sensitive to alkalis and to oxidizing agents, and it is accompanied by chemically similar substances whose presence greatly complicates the problem. It was not long, however, before the isolation was accomplished. In 1934 the independent preparation of the pure corpus luteum hormone was announced from no less than four laboratories. The first public report was made by Butenandt⁷⁹ in an address delivered at Wiesbaden in April, and the details of his work with Westphal and Hohlweg⁷⁴ were published in September. The Butenandt research group isolated two physiologically active diketones, m. p. 128.5° and 120°, together with an inactive hydroxyketone, m. p. 194°. In July, Slotta, Rueschig and Fels⁸⁰ described the preparation from corpus luteum extract of closely corresponding substances, as shown by the melting points: 128°, 119°, 186°. In August, Allen and Wintersteiner⁸¹ announced the isolation,⁸² in the laboratories of the Universities of Rochester and Colum-

⁷⁵ W. M. Allen, *Am J Physiol*, 92, 174, 612 (1930), 100, 650 (1932), W. M. Allen and R. K. Meyer, *ibid.*, 106, 55 (1933).

⁷⁶ W. M. Allen, *J Biol Chem.*, 96, 591 (1932).

⁷⁷ Fevold, Hurw and Leonard, *J Am Chem Soc.*, 54, 254 (1932), Fevold and Hisaw, *Proc Soc Exptl Biol Med.*, 29, 620 (1932).

⁷⁸ Fels and Slotta, *Proc II Internat Congr for Sex Research*, p. 361, London (1930); *Zentr Gynaekol.*, 55, 2765 (1931).

⁷⁹ Butenandt, *Verhandl deutsch Ges innere Med*, Wiesbaden, April 11, *Wien Klin Wochschr.*, 50, 934 (1934).

⁸⁰ Slotta, Rueschig and Fels, *Ber.*, 67, 1270 (1934). See also Slotta and Rueschig, *Z physiol Chem.*, 228, 207 (1934).

⁸¹ W. M. Allen and Wintersteiner, *Science*, 80, 190 (1934).

⁸² Wintersteiner and W. M. Allen, *J. Biol Chem.*, 107, 321 (1934).

bia, of preparations melting at 128°, 121° and 190°. Hartmann and Wettstein,⁸⁵ working in the laboratory of the Gesellschaft für Chemische Industrie, Basel, published in July an account of the isolation of a crystallizate, m. p. 175-177°, which was taken to be an individual hormone; but they later (October)⁸⁶ recognized that this was an error and succeeded in obtaining pure compounds melting at 129°, 120°, and 197°.

While the observations of the various investigators were remarkably concordant, there was at first some difference of opinion regarding the relationship between the two active diketones. The higher-melting substance crystallizes in the form of prisms, the other diketone forms needles, and it was evident that the substances are closely related in chemical behavior. The two compounds give the same derivatives, and although at one time doubts were expressed⁸⁵ regarding the composition of the lower-melting substance, the various workers were soon in agreement in assigning to the two diketones the same empirical formula: $C_{21}H_{30}O_2$. The only reported difference, other than in crystalline form and melting point, was with respect to the physiological activity. Slotta and his collaborators,⁸⁶ who called the compounds "luteosterone C" and "luteosterone D," stated repeatedly that the substances differ decidedly in physiological potency and in the nature of their action, and they regarded them as distinct chemical individuals. This observation however was not confirmed by other workers. Butenandt and Schmidt⁸⁷ reported that in a series of qualitative and quantitative tests the two substances were practically identical. Using the Claiberg test, they (with Hohlweg) found the activity of either diketone to be one rabbit unit per 0.5-0.75 mg. Wintersteiner and Allen,⁸² using the Corner-Allen test, found the activity of both compounds to be the same within the limits of experimental error and placed the minimal dose at 0.6-1.0 mg. Both the Danzig and the American investigators were inclined to believe from the start that the two crystallizates are simply polymorphic modifications of the same substance, and the excellent agreement in their bio-assays by two different methods removed all grounds for the contrary view. The ready interconversion of the two forms⁸³ also speaks for polymorphism. A change sometimes occurs on the resolidification of a melt, but it is best accomplished by crystallization. For example the prism form, m. p. 128°, separates when a solution in hot aqueous alcohol is allowed to cool very slowly,

⁸⁵ Hartmann and Wettstein, *Helv. Chim. Acta*, **17**, 978 (1934)

⁸⁶ *Idem*, *ibid.*, **17**, 1465 (1934)

⁸¹ Neubauer, *Ber.*, **67**, 1627 (1934)

⁸² Slotta, Ruschig and Fels, *ibid.*, **67**, 1621 (1934), Slotta, Ruschig and Blinks, *ibid.*, **67**, 1917 (1934).

⁸⁷ Butenandt and Josef Schmidt, *ibid.*, **67**, 2088 (1934)

⁸³ Fels, Slotta and Ruschig, *ibid.*, **67**, 1621, 1917 (1934), Butenandt and Josef Schmidt, *ibid.*, **67**, 1901, 2044 (1934), Fornsals, *ibid.*, **67**, 2027 (1934)

while the needle form, m. p. 121° , can be obtained by crystallization from pure, dry petroleum ether.

It is clear that, within present knowledge, there is only one corpus luteum hormone. Fortunately the matter of assigning a specific name was the subject of little dispute. Slotta at first used the name luteosterone for the progestational hormone. Allen⁷⁵ (1930) had used the term progestin in connection with his purified extracts, and on isolating the hormone in a pure condition he suggested that the name be retained. An agreement was soon reached between these and other interested investigators in the summer of 1935.⁸⁹ The name progesterone was adopted for the pure hormone, the higher-melting and lower-melting polymorphic forms receiving the specific designations α -progesterone (m. p. 128°) and β -progesterone (m. p. 121°), respectively. The term progestin should be used only in a general sense somewhat similarly to oestrin.

Although progesterone has been detected in the placenta⁹⁰ and in pregnancy urine,⁹¹ these sources do not appear to be practical, and all of the above investigators used as the starting material corpus luteum tissue from sow ovaries. An advantage of this source is that the ovary of the sow contains several corpora lutea, whereas with other available animals, as with humans, each ovary contains but one corpus luteum. The ovaries from a single sow weigh about 12 g., and on cutting out the corpora lutea about 3 g. of yellow-body tissue can be obtained. Since this amount of tissue yields only about 0.08 mg. of the hormone in a crude condition, it is clear that a great many animals are required. The preparation of a purified extract for Butenandt's work was carried out by W. Hohlweg at the Schering-Kahlbaum laboratory, Berlin. About 625 kg. of ovaries from 50,000 sows yielded 12.5 g. of an extract which was active in doses of 3.5-5.5 mg. The pure progesterone (prism form) isolated from this extract amounted to only 20 mg.¹ There are many losses in the final stages of purification; but considering only the purified extract, it may be said that 10-15 sows are required to produce enough hormone to cause the characteristic physiological changes in the uterus of a single rabbit!

The procedures developed by Allen⁷⁵ (1930) for the preparation of purified extracts were largely employed by all of the investigators, as was his method for the removal of follicular hormone (1933). The lengthy but effective processes involve solvent extraction and various partitions between solvents. On distribution between petroleum ether

⁷⁵ W. M. Allen, Butenandt, Corner and Slotta, *Scriber*, **82**, 153 (1935), *Ber.*, **68**, 1746 (1935); *Nature*, **136**, 303 (1935).

⁸⁹ Adler, de Fremery and Taubk, *Nature*, **133**, 293 (1934).

⁹⁰ de Fremery, Luchs and Taubk, *Arch. ges. Physiol.*, **231**, 311 (1932), Loewe and Voss, *Schwens. med. Wochenschr.*, **64**, 1019 (1934).

and 33% alcohol, the corpus luteum hormone is found in the former layer while oestrin concentrates in the latter. After reaching a stage at which a concentration of one rabbit unit per 4-5 mg. has been attained, practically all of the active material can be precipitated as the very sparingly soluble semicarbazone. Following hydrolysis, further purification can be accomplished to advantage by sublimation in high vacuum, by selective adsorption, and by crystallization. The inactive hydroxyketone, m. p. 194°, accompanies the hormone through all of these processes and it is best removed as the acetate, which is distinctly less soluble in alcohol than the hormone.

Progesterone is very sparingly soluble in water and readily soluble in the usual organic solvents. The pure material is colorless and does not deteriorate on storage. Some of the properties are indicated in the table.

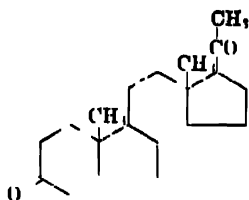
PROPERTIES OF PROGESTERONE

Formula	M p	$[\alpha]_D$	Physiol activity, 1 rb. u.	Absorption maximum	Dioxime, m. p
$C_{21}H_{30}O_2$	Prisms, 128° Needles, 121°	+ 192°	0.5- 1.0 mg.	240 m μ	243°

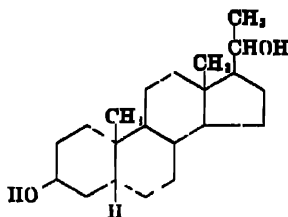
The Structure of Progesterone. The observation that progesterone ($C_{21}H_{30}O_2$) and the inactive hydroxyketone ($C_{21}H_{34}O_2$) differ in composition only by four hydrogen atoms suggested that the latter is a hydrogenation product of the former, or that the two substances have a common origin, and that they are closely related in structure. A further obvious inference was that the substances bear some structural relationship to pregnanediol ($C_{21}H_{34}O_2$), for this also contains twenty-one carbon atoms. The inactive hydroxyketone and pregnanediol indeed yield on oxidation saturated diketones of the same composition. The non-identity of these products was correctly interpreted as indicating that the substances are structurally related but belong to different stereochemical series. Progesterone is an unsaturated diketone, and it was inferred⁸² from the position of the ultraviolet absorption band, in consideration of the work of Menschik, Page and Bossert (page 152), that the ethylenic linkage occupies an α,β -position with respect to a carbonyl group. A sterol-like structure was confirmed by X-ray studies of Neuhaus,⁸⁵ working with the Slotta group, and on the basis of these limited, but most suggestive observations, Slotta and his collaborators⁸³ proposed a formula for the hormone which very shortly was proved to be correct. Indeed,

⁸² Slotta, Ruchig and Fels, *Ber.*, 67, 1624 (1934); Wintersteiner and W. M. Allen, *J. Biol. Chem.*, 107, 321 (1934).

⁸³ Slotta, Ruchig and Fels, *Ann. Wechsch.*, 18, 1207 (1934); *Ber.*, 67, 1624 (1934).



Progesterone



Pregnandiol

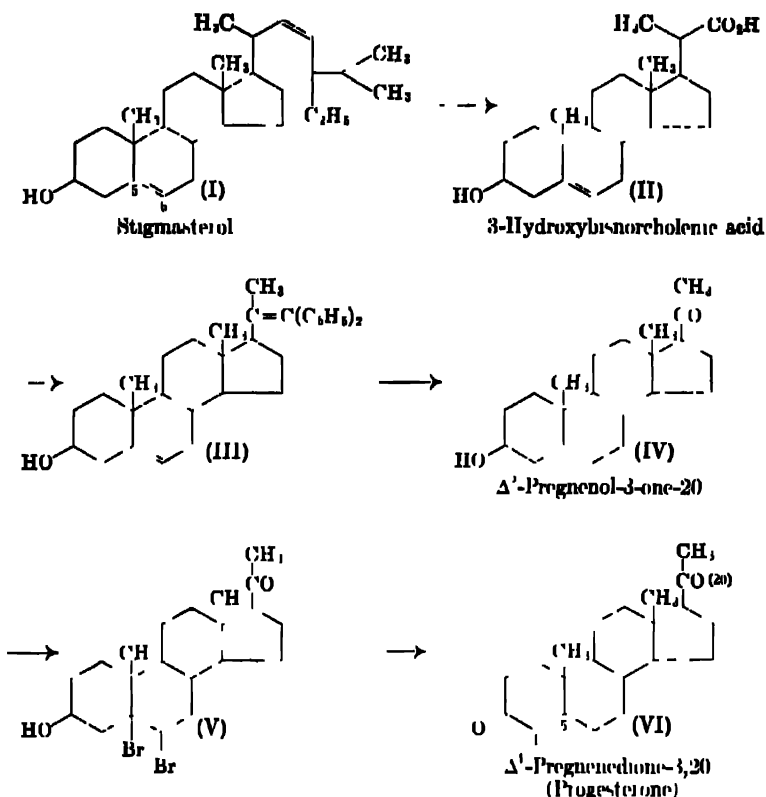
by November, 1934, a complete proof was available of the structure of a substance whose isolation had been reported in April of the same year. Because the amounts of the hormone available were extremely small, the preparative route was chosen for the investigation of the structural problem.

Preparation of the Hormone from Other Natural Products. The timely work of Fernholz⁹⁴ on stigmasterol opened one avenue of approach which both Fernholz and Butenandt were quick to utilize. In his investigation of the phytosterol from soy bean oil (page 170), Fernholz had ozonized the acetate of stigmasterol 5,6-dibromide and obtained, after debromination and hydrolysis, the unsaturated acid II. Butenandt, Westphal and Cobler⁹⁵ converted the ester of II through the diphenyl carbinol into the unsaturated diphenyl derivative III. In order to oxidize III at the newly-formed center of unsaturation without attacking the double bond at C₅-C₆, the latter linkage was protected by the addition of bromine. After acetylation, oxidation, debromination, and hydrolysis, the unsaturated hydroxyketone IV was obtained. The conversion of the secondary alcoholic group of this substance into a ketonic group should give an unsaturated diketone having the composition of the hormone, but at first attempts to oxidize IV gave only mixtures, although a definite physiological activity was detected in the unhomogeneous products. It was soon found,⁹⁶ however, that the pure oxidation product is easily obtained when the unsaturated compound IV is converted into the 5,6-dibromide, V, prior to oxidation. On removing the bromine atoms with zinc dust, a diketone was obtained which was identical in every respect with natural progesterone. In view of the evidence that progesterone contains an α,β -unsaturated system, it is evident that on the regeneration of the double linkage from the dibromide the bond does not appear at the original C₅-C₆ position but in the conjugated position C₄-C₅. A similar shift from the β,γ - to the α,β -position has been established in the case of the dehydrogenation of cholesterol to cholestenone (page 115). By

⁹⁴ Fernholz, *Ann.*, **507**, 128 (1933)

⁹⁵ Butenandt, U. Westphal and Cobler, *Ber.*, **67**, 1811 (1934)

⁹⁶ Butenandt and Josef Schmidt, *ibid.*, **67**, 1001 (1934); Butenandt and U. Westphal, *ibid.*, **67**, 2085 (1934).



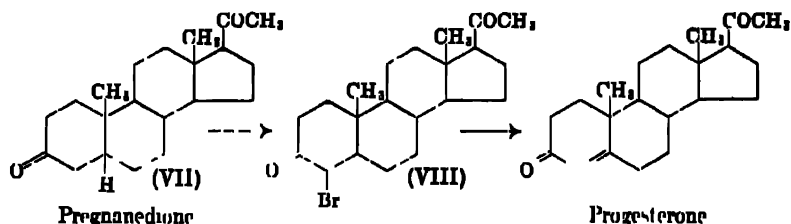
a similar method, namely by heating the substance with copper oxide, Butenandt and co-workers converted the unsaturated hydroxyketone IV directly into the hormone VI. This work completely establishes the structure of progesterone as that of Δ^1 -pregnenedione-3,20 (VI). To fit the optical evidence, the double bond must be at either C₁-C₂ or at C₁-C₂, and since in stigmasterol it is joined to C₅ (and C₆) a shift to the former position is understandable and a migration to the other side of the ring, to C₁-C₂, is excluded.

At practically the same time as the work of the Butenandt group was announced, Fernholz⁹⁷ reported the independent synthetic preparation of progesterone from stigmasterol by exactly the same methods. The results are in excellent agreement with those of Butenandt.

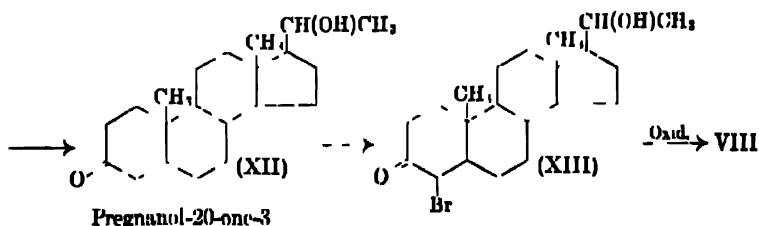
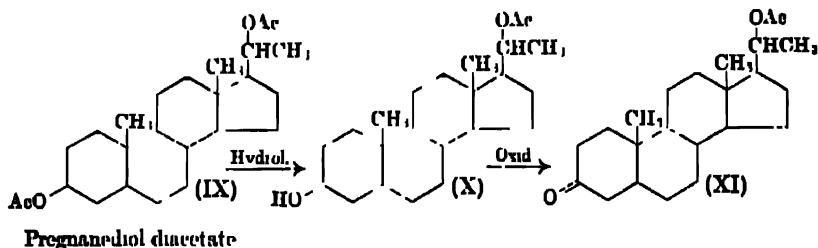
In a paper antedating by a slight margin the complete accounts of the above work, Butenandt and Schmidt⁹⁸ described the production of pure progesterone by another method of pseudo-synthesis. Starting with pregnanediol from human pregnancy urine, the saturated diketone (VII)

⁹⁷ Fernholz, *Ber.*, 67, 1653, 2027 (1934)

obtained on oxidation was brominated and the product (VIII) was treated with pyridine to effect the elimination of hydrogen bromide. The unsatu-



rated diketone was identical with the corpus luteum hormone. The bromodiketone VIII was also prepared from pregnanediol by a longer series of transformations in which pregnanol-20-one-3 (XII)⁸⁹ was the essential intermediate.

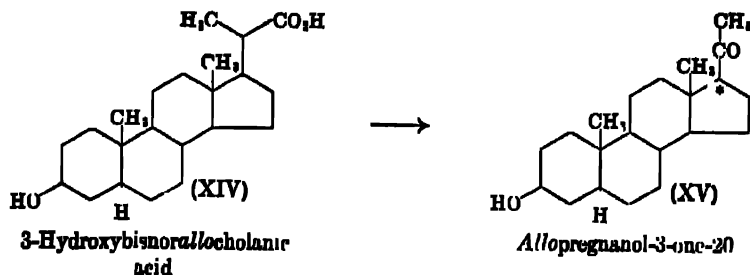


There remains for consideration the structure of the saturated hydroxyketone which is found as a companion of progesterone in extracts of corpora lutea. The problem was solved by the synthetic production of the substance from stigmasterol by Butenandt and Mamoli,⁸⁹ and later by Fernholz.¹ The actual starting point was 3-hydroxybisanorallocholanolic acid (XIV), which Fernholz had obtained by the hydrogenation of 3-hydroxybisanorcholonic acid (page 170). The $>\text{CHCO}_2\text{H}$ group was degraded to $>\text{CO}$ by the usual Grignard procedure, and the product (XV) proved to be identical with the natural hydroxyketone, m. p. 194° . This

⁸⁹ Butenandt and Josef Schmidt, *Ber.*, **67**, 1893 (1934).

⁹⁰ Butenandt and Mamoli, *ibid.*, **67**, 1897 (1934).

¹ Fernholz, *Z. physiol. Chem.*, **230**, 195 (1934).



may well arise in the organism by the hydrogenation of the unsaturated diketone progesterone. Butenandt and Mamoli² later found that *allopregnanol-3-one-20* (XV) is isomerized to a certain extent by alkali into *isopallopregnanol-3-one-20*, a stereoisomer melting at 148°. The change can be in part reversed under the same conditions, and it is interpreted as being due to racemization at the asymmetric carbon atom (C₁₇) in the α -position to the carbonyl group. Both hydroxyketones probably have the same configuration at C' since they are precipitated by digitonin, and each yields a different diketone as the chief product of oxidation. *Isopallopregnanol-3-one-20* gives *isopallopregnanedione* (m. p. 135°) and only a small amount of *allopregnanedione* (m. p. 200.5°).

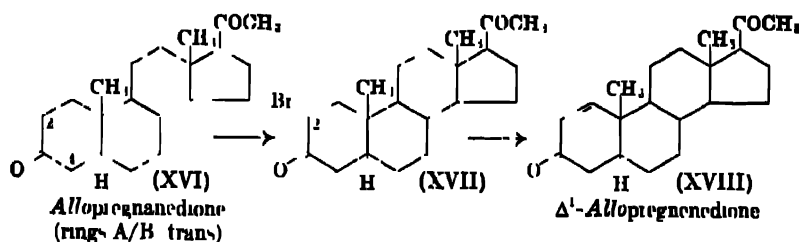
In the course of the above work Butenandt and Mamoli made the striking observation that *allopregnanol-3-one-20* acetate and *allopregnanedione-3,20* (but not the *iso*-compounds) very slowly form characteristic, sparingly soluble digitonides from which the compounds may be regenerated. This is an exception to the rule that acetyl derivatives and ketones from sterol-like alcohols do not form stable molecular compounds with digitonin.

The preparation of the inactive hydroxyketone (XV) of the corpus luteum from 3-hydroxybisanorallocholanic acid proves that the substance belongs to the *allo*-series (rings AB:trans). Androsterone has the same configuration of the ring system, and dihydrocholesterol, which occurs along with cholesterol but which is not absorbable by the organism, is likewise an *allo*-compound. Pregnanediol belongs to the alternate stereochemical series, and pregnanedione (m. p. 123°) differs from the oxidation product of the hydroxyketone (XV) of the corpus luteum (*allopregnanedione*, m. p. 200.5°).

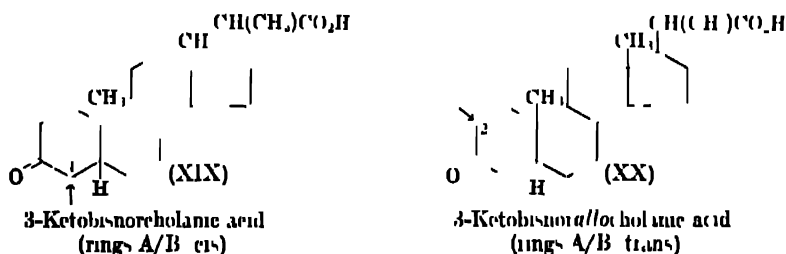
Butenandt and Mamoli³ investigated the bromination of *allopregnanedione* (XVI) with interesting results. Whereas the (AB) *cis* isomer, pregnanedione, gives the 4-bromo derivative, which easily loses hydrogen bromide and yields progesterone, the *alldione* (XVI) is converted

² Butenandt and Mamoli, *Ber.*, **68**, 1817 (1935)

³ *Idem. ibid.*, **68**, 1880 (1935)



into the 2-bromo compound XVII (probable structure). Hydrogen bromide is split from this bromo diketone only with great difficulty (potassium acetate at 180°) and the product is an isomer of progesterone. The new diketone has the absorption spectrum of an α,β -unsaturated ketone and the probable structure is that of Δ^1 -allopregnenedione, XVIII (alternate structure: Δ^{10} -isomer). It is a striking observation that the course of the bromination is dependent upon the stereochemical relationship of rings A and B. To investigate the generality of the reactions, Butenandt and Mainoli⁴ prepared the stereoisomeric keto acids XIX and XX by standard methods and studied their reactions with bromine. Exactly as



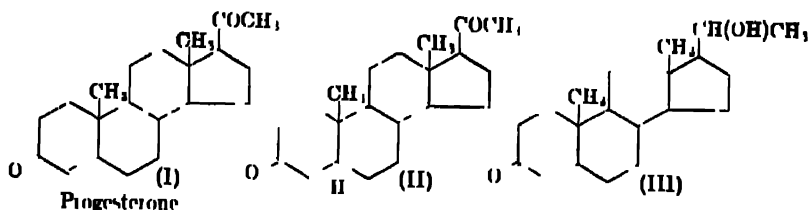
with the diketones, the *cis* compound XIX yields a 4-bromo derivative which easily loses hydrogen bromide, while the *trans* compound XX is brominated at the 2-position and the 2-bromo derivative forms an unsaturated compound with difficulty. Parallel results were obtained with coprostanone and cholestanone.⁵ Evidently the spatial character of the ring system is an important factor in determining the course of such reactions as bromination.

Specificity of the Progestational Hormone. As compared with the other known sex hormones, progesterone is active only in relatively enormous doses. From such results as are available, the pregnancy hormone also seems to differ from the other hormones of the gonads in being highly specific. No other substance has been reported to respond to the Corner-Allen or Clauberg tests. The unsaturated linkage appears to be an essential feature of structure, for neither pregnanedione nor allopregnanedione

⁴ Butenandt and Mainoli, *Ber*, **68**, 1854 (1935).

⁵ Butenandt and Wolff, *ibid.*, **68**, 2091 (1935).

dione has the progestational activity of the hormone (I). The double bond apparently must occupy a specific position, however, for Δ^1 -*allo*-pregnenedione (II) is inactive up to 1 mg. in the Clauberg test. The



various saturated and unsaturated hydroxyketones mentioned in the foregoing discussions are all inactive, as is the (3)-desoxyhormone.⁶ Particularly interesting is the case of the dihydro hormone III, which was prepared from pregnanol-20-one-3 through the 4-bromo derivative.⁷ Although the reduction of the carbonyl group at this end of the molecule gives compounds of enhanced physiological activity in the case of oestrone, androsterone, and androstenedione, both the free alcohol, III, and the acetate appear to be quite inactive.

BIOGENETIC RELATIONSHIPS

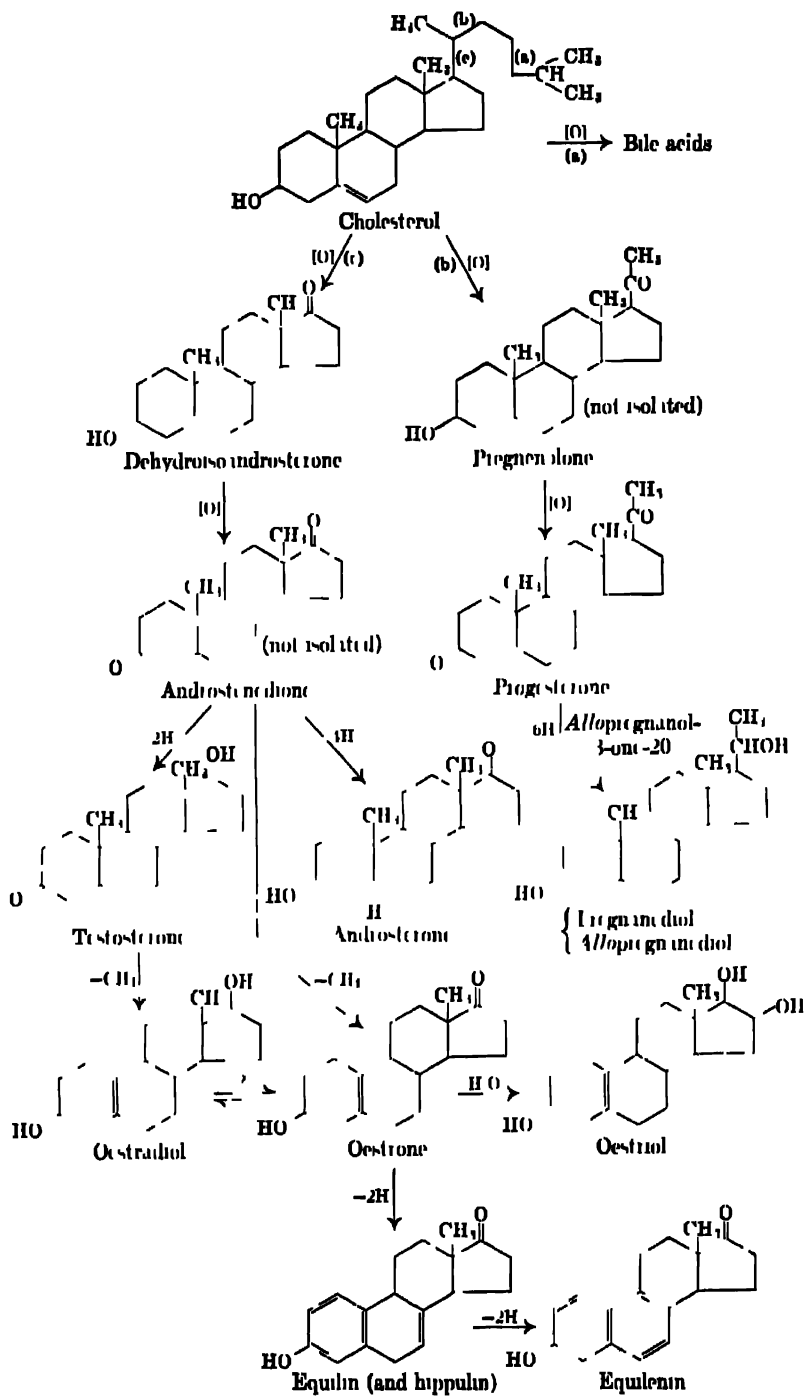
In concluding this survey of the striking developments in hormone chemistry in the six-year period since the isolation of oestrone in 1929, the various indications regarding the probable mode of formation of the hormones from cholesterol are summarized in the chart on page 252. Taken as a whole, the observations as to the structures and companion substances of the different hormones isolated from both tissue and urinary extracts form a remarkably consistent picture. In the first recognized phases of metabolism it appears that the cholesterol side chain is subject to degradation at three different points. The elimination of the isopropyl group by oxidation (a), along with changes in the nucleus, can lead to the bile acids, while a rupture at either the one (b) or the other (c) side of the branching methyl group can afford pregnenolone and dehydroisandrosterone. There is ample experimental analogy for the first and third types of oxidation in the methods employed for the preparation of the cholanic acids and of androsterone from saturated sterol derivatives. Although pregnenolone has not been discovered in the organism and is known only as a synthetically prepared transformation product, Butenandt's suggestion⁸ that it is a probable intermediate in the formation of progesterone is supported by the isolation of an entirely

⁶ Butenandt and U. Westphal, *Ber.*, **67**, 2085 (1934).

⁷ Butenandt and Josef Schmidt, *ibid.*, **67**, 2092 (1934).

⁸ Butenandt, *Deut. med. Wochenschr.*, **61**, 823 (1935).

SEX HORMONES



analogous unsaturated hydroxyketone, dehydroisoandrosterone, from male urine. Significant of the origin of dehydroisoandrosterone is the fact that it corresponds precisely with cholesterol in the location of the double bond and the configuration at C_5 .

In the next step it is supposed that the unsaturated hydroxyketones become oxidized or dehydrogenated to the unsaturated diketones androstenedione and progesterone. There is good experimental analogy for these reactions and indeed the direct dehydrogenation of pregnenolone has been realized in the laboratory (Butenandt). Although androstenedione has not been isolated as a natural product, its existence as an intermediate compound in the metabolic series is strongly indicated by the isolation of the analogously constituted progesterone.

The fate of progesterone in the organism is indicated by the isolation of the reduction products pregnanediol and *allopregnanediol*. Since the diols differ only in the configuration at C_5 , they probably arise from the *cis* and *trans* addition of hydrogen to a C_5 -unsaturated precursor, such as progesterone or pregnenolone. *Allopregnanol-3-one-20*, the inactive saturated hydroxyketone found in the corpus luteum evidently is an intermediate product of the hydrogenation. That pregnanediol and *allopregnanediol* are found only in pregnancy urine is a convincing indication that they come from the hormone which controls pregnancy.

That progesterone suffers hydrogenation in the organism lends considerable support to the hypothesis (Ruzicka, Butenandt) that androstenedione similarly affords testosterone and androsterone by the addition, in independent processes, of two and of four hydrogen atoms, respectively. Androsterone and *allopregnanol-3-one-20* are in the same state of hydrogenation and they are both *allo*-compounds reduced at C_{19} , but they differ in the stereochemical arrangement at the secondary hydroxyl group. Since androsterone is an *epi*-compound having the opposite configuration at C_5 from cholesterol, the hormone cannot arise directly from either cholesterol or dehydroisoandrosterone. The epimerization requires an intermediary phase in which the asymmetry at C_5 has been destroyed by oxidation, as in androstenedione. The existence of this unsaturated diketone as an intermediate would also provide a simple route to testosterone.

The oestrogenic hormones have the same carbon skeleton as the male hormones except for the absence of the characteristic methyl group at C_{10} , and consequently they probably arise either from these substances or from common precursors. For a time it seemed possible that oestrone is a product of the dehydrogenation of androsterone, but animal experiments expected to detect such a conversion have failed to sustain the view. The isolation of testosterone and oestradiol has led to the adoption

anism of oxidation and reduction which appears to be simple and reasonable, views regarding the origin of the hormones have in the past been so subject to rapid change with new discoveries that it is far from certain that the final verdict has been reached.

The many structural relationships among the hormones and between these substances and cholesterol provide abundant evidence of a circumstantial nature that the hormones actually come from cholesterol and do not arise by independent biosynthesis. It would be very difficult to explain in terms of the alternate view the exact correspondence in structure and configuration between dehydroisoandrosterone and cholesterol or to understand why an organism requiring testosterone should also synthesize dehydroisoandrosterone. By inference it appears likely that the bile acids are also products of cholesterol metabolism. The acids of the bile can hardly come from cholesterol through dihydrocholesterol, the normal product of hydrogenation in body tissue, for they belong to the coprostanone rather than to the cholestanone series. The configuration at C_5 also is the opposite of that of cholesterol and dihydrocholesterol, and consequently it is clear that the conversion of cholesterol into the various bile acids would involve both an allomerization and an epimerization, in addition to the shortening of the side chain, the saturation of the molecule, and such further hydroxylation as is required. In analogy with the relationships indicated between the hormones it seems entirely possible that both stereochemical inversions occur through the intermediary of an oxidation product similar to androstenedione and progesterone, namely cholestenone or 3-keto- Δ^4 -choleonic acid. It is significant that ketocholeonic acids have been discovered in bile, and that the biological reduction of coprostanone and of triketocholanic acid has been demonstrated experimentally (Chapter IV).

As for the origin of cholesterol in the body, nothing is known except that the substance is a product of synthesis in the animal organism. Interesting but entirely speculative suggestions have been advanced regarding the possible formation of cholesterol from squalene⁹ or from carotene.¹⁰ The regular appearance of a hydroxyl group at the characteristic C_3 -position not only in the case of the sterols, bile acids, and hormones, but also in nearly all of the natural products described in the succeeding chapters of this book, suggests either a regular building principle or a special characteristic of this position. The greater reactivity of functional groups at C_3 as compared with the same groups at C_7 or C_{12} , suggests that the cholane ring system may be more susceptible to oxidation or hydroxylation at C_3 than elsewhere in the molecule.

⁹ Vangelovich, *Chemistry and Industry*, 53, 905 (1934); R. Robinson, *ibid.*, 53, 1063 (1934); Alincovici, *Bull. soc. chim. biol.*, 17, 366 (1935).

¹⁰ Bryant, *Chemistry and Industry*, 54, 907 (1935).

Chapter VI

Heart Poisons

There are two groups of natural poisons which are characterized by the highly specific and powerful action which they exert on the cardiac muscle when injected intravenously. One type is a product of plant synthesis, while the other is elaborated in the organism of the toad and is found in the skin secretions of the animal. The plant heart poisons are glycosides, and on the hydrolysis of such a substance there is obtained as the sugar-free moiety a hydroxylated polynuclear compound known as a cardiac aglycone or genin. The use of the latter term is based upon the names of some of the members of the group. For example, the glycosidic poison digitoxin yields on hydrolysis an aglycone known as digitoxigenin. The characteristic cardiac-stimulating, or cardiotonic, quality of the poison is derived from the special structure of the aglycone part of the molecule and the sugar group or groups have only a modifying influence. The toad poisons also contain in the molecule a hydroxylated polynuclear moiety conjugated not with sugars but with suberylarginine. It is now known that the two types of genins are related in structure as well as in their physiological properties.

It was only after extensive investigation that the true chemical nature of these complicated natural products became apparent, and the picturesque names assigned to the compounds reflect their origin or their characteristic physiological property but have no chemical significance. As a consequence the terminology employed in the literature for the description of derivatives and transformation products is unavoidably cumbersome.

CARDIAC GLYCOSIDES

As stated above, the poisons of this group have in common a characteristic influence on the activity of the heart. Very small doses of these materials can exert a beneficial action on the diseased organism by stimulating the heart to greater contractional activity and thus quickening the pulse; too large doses lead to severe injury and eventually to the stoppage of the heart in systole (condition of contraction). The active principles occur in various plants having a wide geographical distribution, particularly those of the order *Apocynaceae*. Others have been found in the *Scrophulariaceae*, *Iliaceae*, and *Ranunculaceae*. Many species grow

in tropical regions and have been used as a source of arrow poisons. Digitalis plants, such as the foxglove, were used in the preparation of poisons for the medieval ordeals, and drugs made from the dried leaves of the plants have long been used as remedies. Digitalis was first employed for the treatment of dropsy in 1785 by the English physician William Withering, and the use of the drug in heart therapy has met with some spectacular successes. In the words of Cushny,¹ "digitalis has long been the sheet-anchor in treatment of diseases of the heart." The pharmaceutical preparations of digitalis come from the seeds and leaves of *Digitalis purpurea* (purple foxglove). The sea onion or squill (*Scilla maritima*), a bulbous herb of southern Europe and northern Africa, was used as a medicine by the ancient Egyptians and Romans. It has been employed as an expectorant, a cardiac stimulant, a diuretic, and a rat poison. *Strophanthus* species, such as *Strophanthus kombé*, *S. hispidus*, and *S. gratus*, have been used in tropical Africa in the preparation of arrow poisons, the drug being obtained from the seeds and bark. Certain African tribes have employed the root of the uzara as a drug (uzaron). Some idea of the great potency of these substances is given by the fact that 0.07 mg. of strophanthin is sufficient to cause the heart of a 20 g. mouse to stop beating within a few minutes after injection.

The chemical investigations of the active principles of the digitalis plants date from the early part of the nineteenth century (Destouches, 1808; Homolle and Quevenne, 1842). In 1869 Nativelle for the first time isolated a crystalline glycoside (digitalin) in a fairly pure condition. In the following years the chief contributions to the problem of isolation were made by German chemists, particularly by Schmiedeberg, Cloetta, Killiani, and Krafft. The work of Windaus, undertaken in 1915, furnished the first insight into the structural character of the physiologically active substances, and the extensive investigations (1922-1934) of W. A. Jacobs of the Rockefeller Institute, New York, provided a sound foundation for the eventual solution of the major problems of structure.²

The chemistry of the cardiac glycosides presents points of extraordinary complication, as will be appreciated from the discussions given below, and materials suitable for investigation are by no means easily obtained. The drugs are rare and expensive, they often are of variable character, and the poisonous principles are present in very small quantities. Several physiologically active and closely related substances often occur together in the same plant, and the inactive saponins, digitonin and

¹ A. Cushny, "Pharmacology and Therapeutics," 10th Ed., revised by C. W. Edmunds and J. A. Gunn, pp. 475-507 (1934). See also Cushny, "Digitalis and its Allies," Longmans, Green and Co., London (1925).

² For a survey of the early work, see W. A. Jacobs, "The Chemistry of the Cardiac Glycosides," *Physiol. Rev.*, 13, 222 (1933).

gintonin, are frequently present and are extracted along with the cardio-tonic substances. It is usually a matter of great difficulty to carry the purification beyond the stage of an amorphous mixture of related compounds, or of mixed crystals. The plant heart poisons are all glycosides, and some of them are extremely sensitive to the hydrolytic action of acids, bases, or enzymes. The "melting points" are really points of decomposition and they are only roughly characteristic and afford no sure indication of identity or purity.

The methods of extraction and purification vary with the source and with the special properties of the individual compounds. The glycosides are all soluble in water and alcohol and sparingly soluble in non-hydroxylic solvents. The dried and powdered seeds, leaves, or roots often are first extracted with ether or ligroin in order to remove fats and resins. By exhaustive extraction with methanol or with 70% ethanol the glycosides are brought into solution, leaving a residue consisting largely of cellulose. The alcoholic solution is evaporated in vacuum to a thick syrup and this is taken up in warm water. A crude glycoside mixture often separates at this point if the solution is allowed to stand for several days, but it usually is advisable to introduce other purification steps. Saponins can be precipitated by the addition of basic lead acetate, and the excess reagent is then eliminated from the filtrate by the addition of either sulfuric acid or sodium phosphate. Extraction of the aqueous solution with ether or chloroform removes tars without the loss of physiologically active material from the water layer. After clarification by these methods, the glycosides can be caused to separate by saturating the water solution with solid ammonium sulfate.

For use as pharmaceuticals, it is desirable to obtain the cardiac glycosides in the condition in which they occur in the plant, but the genins or aglycones obtained on the hydrolysis of the glycosides are of much more importance to the problem of structural determination. Treatment with hydrochloric acid effects the complete hydrolysis and gives an aglycone and one or more molecules of a sugar, or sugars. The more important glycosides, together with their products of hydrolysis, are listed in the table. It should be noted that two or more glycosides may yield the same genin. *k*-Strophanthin (purest form: *k*-strophanthin- β), the chief glucoside from *Strophanthus kombé* seeds, is cleaved to strophanthidin, glucose, and cymarose:



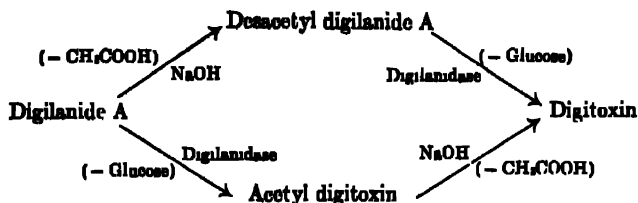
Cymarín, from Canadian hemp,² yields the same genin and cymarose:

² Windaus and Hermanns, *Ber.*, 48, 979, 981 (1915).

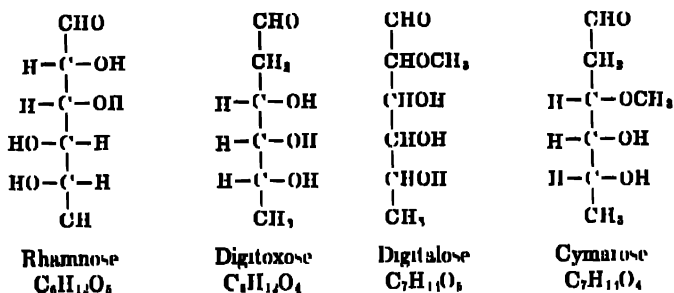
CARDIAC GLYCOSIDES AND AGLYCONES

Glycoside	Formula	M p	Aglycone	Formula	M p	Sugar
k-Strophanthin- β	$C_7H_{10}O_{11}$	175°	Strophanthidin ^a	$C_7H_8O_6$	175°	Glucose + Cymarose ($C_7H_{10}O_6$)
Cymarine	$C_7H_8O_{10}$	138°	Periplogenin	C_7H_8O	185°	Cymarose
Periplocin	$C_7H_8O_{10}$		Sarmentogenin ^a	$C_7H_8O_6$	266°	Hexose + Cymarose Sarmentose ($C_7H_8O_6$)
Sarmentocymarine	$C_7H_8O_{10}$		Digitoxigenin ^a	$C_7H_8O_6$	250°	3 Digitoxose ($C_6H_8O_6$) + Glucose + Acetic acid
Diglanide A	$C_7H_8O_{10}$	245°				3 Digitoxose
Digitoxin	$C_{25}H_{40}O_{11}$	263°				3 Digitoxose + Glucose + Acetic acid
Diglanide B	$C_7H_8O_{10}$	245°	Gitoxigenin ^a	$C_7H_8O_6$	235°	3 Digitoxose
Gitoxin	$C_{25}H_{40}O_{11}$	285°				Glucose + Digitalose ($C_7H_8O_6$)
Digitalin	$C_7H_8O_{10}$	229°	Dianhydrodigitoxigenin ^a	$C_7H_8O_6$	213°	Digitalose
Oleandrin	$C_7H_8O_{10}$	249°	(Digitalactin)			3 Digitoxose + Glucose + Acetic acid
Diglanide C	$C_7H_8O_{10}$	248°	Digitoxigenin ^a	$C_7H_8O_6$	222°	3 Digitoxose
Digoxin	$C_7H_8O_{10}$	265°				2 Glucose
Uzarin	$C_7H_8O_{10}$	270°	α -Anhydro-uzarigenin ^a	$C_7H_8O_6$	265°	Rhamnose
Orobanchin	$2H_2O$	188°	(Orobanchin) ^a	$C_7H_8O_6$		Glucose + Rhamnose
Scillaren A	$C_7H_8O_{10}$	270°	Scillaridin A ^a	$C_7H_8O_6$	250°	

- ^a Jacobs and Hendelberger *J Biol Chem* 54, 253 (1922) Jacobs *ibid.* 57, 583 (1923) Jacobs and Hoffmann *ibid.* 67, 608 (1926) 69, 163 (1926)
- ^b Jacobs and Hoffmann *J Biol Chem* 79, 319 (1928) Lehmann *Arch Pharm* 235, 157 (1957)
- ^c Jacobs and Hendelberger *J Biol Chem* 81, 765 (1929)
- ^d Cloetta *Arch exp Path Pharmacol* 88, 113 (1920) Windaus and Freese *Ber* 56, 2503 (1923) The correct formula was established by Windaus, *Nach Ges Wiss Göttingen* 170 (1928)
- ^e Windaus and Schwartz *Ber* 56, 1215 (1923) The correct formula was suggested by Jacobs and Gustus *J Biol Chem* 78, 573 (1928) and established by Windaus K. Westphal and Stein *Ber* 61, 1847 (1924)
- ^f Windaus and Baandte *Ber* 56, 2001 (1923) Windaus K. Westphal and Stein *loc cit*
- ^g Smith *J Chem Soc* 503 2478 (1930) 23 (1931) 1303 (1935)
- ^h Windaus and Haack, *Ber* 63, 1377 (1930)
- ⁱ Jacobs and Bigelow *J Biol Chem* 96, 647 (1932) 101, 15 (1933)
- ^j Stoll Suter Kren Busermaker and A. Hofmann *Helv Chim Acta* 16, 703 (1933)



With the exception of glucose and rhamnose, the sugars resulting from the hydrolysis of the plant poisons have not been found elsewhere. Digitoxose was recognized by Kilianni (1922) as a 2,6-deoxyhexose and the configuration was established by Micheel.¹⁶ Digitalose is probably the 2-methyl ether of a methyl pentose (Kihani). Cymarose was regarded



by Windaus and Hermanns¹⁷ as a monomethyl ether of digitoxose. Elderfield¹⁸ established this relationship and located the ether group at C_2 . Jacobs and Bigelow¹⁹ consider sarinentose ($\text{C}_7\text{H}_{14}\text{O}_4$) to be the methyl ether of a desoxy sugar. When a genin is linked to a desoxy sugar, hydrolysis of the glycoside occurs with great readiness. Other sugars can be detached from the genin nucleus only under conditions of hydrolysis so drastic as to promote secondary changes. The secondary change usually consists in the splitting off of one or more molecules of water from the genin with the formation of a mono-, di- or tri-anhydro compound according to the number of alcoholic hydroxyl groups eliminated. For example the true genin from uzarin has not yet been obtained, for under the conditions of the acid hydrolysis a molecule of water is eliminated with the formation of anhydro-uzarigenin. Ouabain has not yet yielded a genin, although it is possible from other evidence to infer the composition of the hypothetical ouabagenin. In this case the secondary changes are more profound (loss of one carbon atom).

¹⁶ Micheel, *Ber.*, 63, 347 (1930)

¹⁷ Windaus and Hermanns, *ibid.*, 48, 879 (1915)

¹⁸ Elderfield, *J. Biol. Chem.*, 111, 527 (1935)

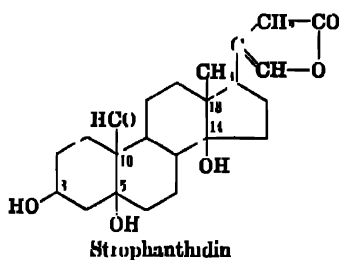
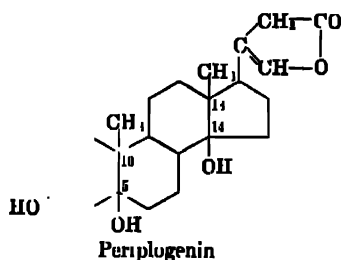
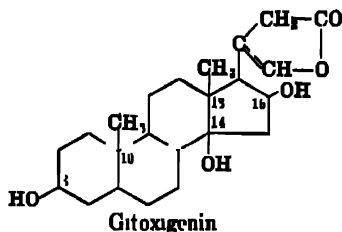
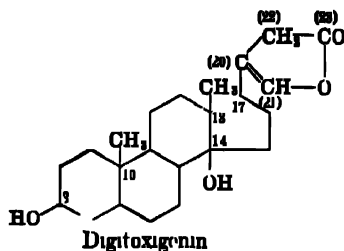
¹⁹ Jacobs and Bigelow, *ibid.*, 96, 355 (1932).

The ready dehydration of the genins under the influence of mineral acids has been the source of some confusion. The glycosides digitalin and oleandrin were found to yield on hydrolysis the same aglycone moiety ($C_{23}H_{30}O_3$), and this was called "digitaligenin" and regarded as a true genin. Windaus and Schwarte⁸ later obtained the same substance by heating gitoxigenin ($C_{23}H_{34}O_3$) with alcoholic hydrochloric acid, and they were able in this way to recognize the supposed genin as dianhydro-gitoxigenin.

The Structures of the Aglycones. The determination of the structural formulas of the genins was an arduous and perplexing problem, and there was no adequate working hypothesis concerning the nature of the complex molecules to guide the work which paved the way for the eventual solution. Windaus contributed much to the development of the field, but the foundations of the chemistry of the cardiac aglycones were worked out largely in the laboratory of W. A. Jacobs. Following the developments in the sterol-bile acid field, the cardiac substances were finally related to these other natural products in 1934-1935, and the information which had accumulated from the earlier, "blind" work was so extensive and so accurate that the complete structures of the more important aglycones could be inferred by accommodating these data to a sterol-like ring system. In this way the major problems were soon solved. The final unravelling of the structures was the work of W. A. Jacobs and R. C. Elderfield, of R. Tschesche in Windaus' laboratory, and of A. Stoll. Without making experimental observations of his own, G. A. R. Kon of the Imperial College, London, contributed to the problem by reviewing the early work in terms of the newly established carbon skeleton of the sterols.²⁰

From the formulas given in the table on page 259 it will be seen that with the one exception of scillaridin A, the cardiac aglycones are C_{23} -compounds, mainly of the formula $C_{23}H_{34}O_{(4-5)}$. Scillaridin A possesses certain special features of structure and it will be considered separately, after reviewing the chemistry of the aglycones of the digitalis-strophanthus-uzara group. The structures of four of these substances are now known with a close approach to certainty, and it will facilitate the discussion to employ the accepted structures in surveying the evidence on which they are based. The simplest substance of the group is digitoxigenin, and the striking relationship to the sterols is at once apparent from the formula. There is a secondary hydroxyl group at C_3 , angular methyl groups are located at the usual 10- and 13-positions, and the car-

²⁰ Review articles: Tschesche, *8 angew Chem*, 47, 720 (1934); *8 physiol Chem*, 229, 219 (1934); Jacobs and Elderfield, *J. Biol. Chem.*, 108, 497 (1935); Kon, *Chemistry and Industry*, 53, 693, 656 (1934); Kon, *Chemical Society Annual Reports*, 31, 219-238 (1935); Elderfield, *Chemical Reviews*, 17, 187 (1935).

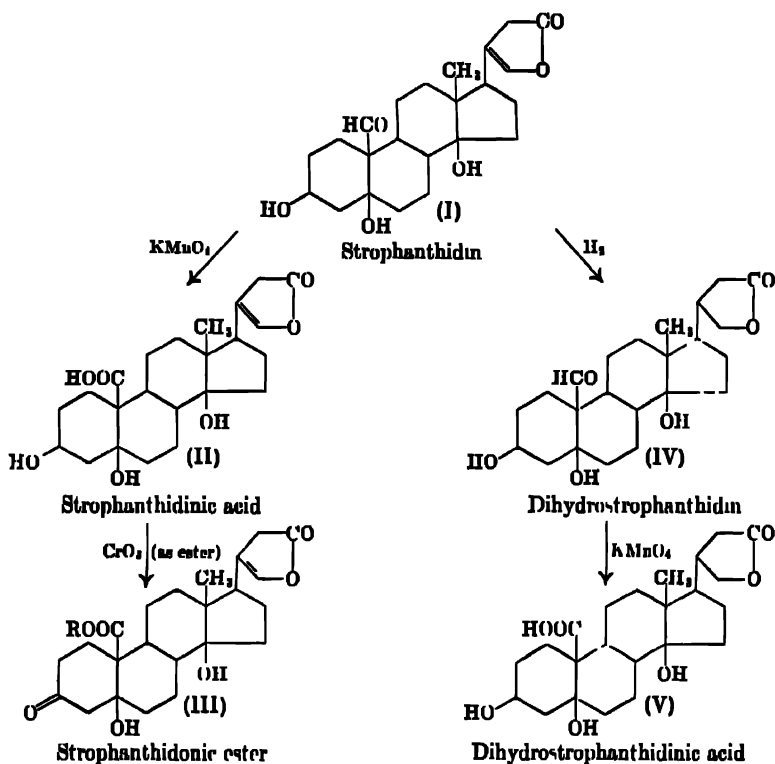


bon atoms of the lactone ring at C₁₇ conform to the pattern of this part of the sterol side chain, there being one carbon atom less than in a bile acid. The unsaturated lactone ring is perhaps the most distinctive feature of structure, and in addition there is in digitoxigenin a tertiary hydroxyl at C₁₄. Gitoxigenin differs only in having an additional (secondary) hydroxyl group at C₁₀ in the five-membered ring. Periplogenin is isomeric with gitoxigenin, the hydroxyls being located at C₃, C₇, and C₁₄. The number and arrangement of the hydroxyl groups is exactly the same in the strophanthidin molecule, but this substance is unique in having an aldehydic group at C₁₀ instead of the usual methyl group. Because of the extra functional group, strophanthidin is capable of undergoing a greater variety of transformations than the other aglycones, and the chemistry is more complicated. Most of the work of Jacobs and his collaborators was on strophanthidin and, as events have shown, the greater opportunity for manipulating the molecule eventually furnished indispensable information, although for a time the multifold transformations presented a most perplexing problem.

Strophanthidin: the Functional Groups. The aldehydic character of strophanthidin was not at once apparent. Since the aglycone forms an oxime but does not reduce Fehling's solution²¹ it seemed possible that the carbonyl group is present in a ketonic linkage. The aldehydic nature of the group was shown by its conversion to a carboxylic acid group on oxidation of the aglycone with permanganate in acetone solution to

²¹ Jacobs and Collins, *J. Biol. Chem.*, **65**, 491 (1925)

strophanthidinic acid (II), which contains one more oxygen atom than strophanthidin (I).^{21,22} It is worthy of note that although the secondary alcoholic group at C₃ and the double bond in the lactone ring are



vulnerable points of attack by oxidation, only the aldehydic group is affected by neutral permanganate. Once this change has been accomplished, the secondary alcoholic group can be oxidized with the use of chromic anhydride, as shown by the conversion of strophanthidinic ester to strophanthidonic ester (II→III).²³ Strophanthidin forms a monoacetate and a monobenzoate, and since strophanthidonic ester (III) forms no such derivatives, the observations identify the hydroxyl group of strophanthidin responsible for the formation of acyl derivatives as a secondary alcoholic group.

Of the two unsaturated centers in strophanthidin, the first to be attacked on catalytic hydrogenation is the double bond in the lactone ring, the product being the dihydro derivative, IV.²⁴ That the aldehydic

²¹ Jacobs, *J. Biol. Chem.*, **57**, 556 (1923), at first assigned to the acid ($\text{C}_{20}\text{H}_{32}\text{O}_7$) the formula $\text{C}_{20}\text{H}_{32}\text{O}_7$.

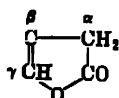
²² Jacobs and Gustus, *ibid.*, **74**, 795 (1927).

²³ Jacobs and Heidelberg, *ibid.*, **54**, 253 (1922).

group is still intact is shown by oxidation of the dihydro compound to an acid, V, with permanganate in acetone solution. The resistance of the aldehydic group to reduction is further shown by the fact that it is only with great difficulty that dihydrostrophanthidin (IV) can be converted by catalytic hydrogenation into the corresponding $-\text{CH}_2\text{OH}$ compound.²⁵ The lack of reactivity of the aldehydic group doubtless is due to steric effects, for the group is attached to a quaternary carbon atom.

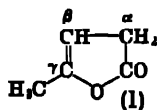
Strophanthidin reduces Tollens' reagent in pyridine solution, and since dihydrostrophanthidin does not respond to the test it can be concluded that it is the unsaturated lactone ring and not the aldehydic group which is responsible for the observed effect. This is one reason for placing the double bond in the lactone ring, the presence of which is demonstrated by titration experiments. The presence of two tertiary alcoholic groups is best demonstrated by their elimination under dehydrating conditions, as will be described later.

The Lactone Ring:

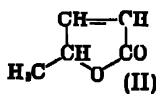


In addition to the test with

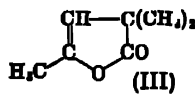
Tollens' reagent, much use has been made of a color reaction discovered by Legal in 1883.²⁶ A pyridine solution containing strophanthidin and sodium nitroprusside rapidly acquires a deep red color on adding a few drops of alkali.²⁷ Dihydrostrophanthidin does not give the nitroprusside reaction, and the Legal test is negative with all other derivatives or transformation products which no longer contain the double bond. The test, on the other hand, is characteristic of all of the aglycones of the digitalis-strophanthus group, and of their lactone-unsaturated derivatives. In model experiments with the angelica lactones, Jacobs, Hoffmann and Gustus²⁸ found that the color reaction is typical of β,γ -unsaturated γ -lactones having an α -hydrogen atom (I). The test is negative when the



Positive Legal test



Negative



Negative

double bond is hydrogenated, or when it is transposed to the α,β -position (II). The lactones which respond to the test also have an active hydrogen atom, as shown by the Zerewitinoff determination, probably because of enolization from the α -position. On replacing both α -hydrogen

²⁵ Jacobs, *J. Biol. Chem.*, **85**, 519 (1930).

²⁶ Hans Meyer, *Analyse u. Konstitutionsermittelung*, 5th Ed., p. 446 (1931).

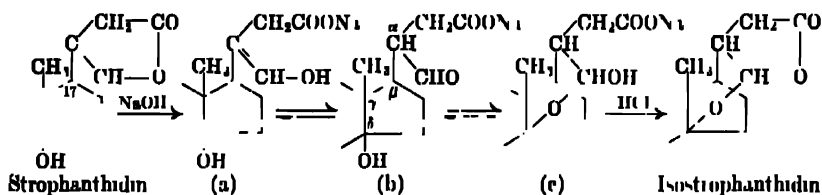
²⁷ Jacobs and Hoffmann, *J. Biol. Chem.*, **67**, 333 (1926).

²⁸ Jacobs, Hoffmann and Gustus, *ibid.*, **70**, 1 (1926).

atoms with alkyl groups (III) both the tests of Legal and of Zerewitinoff are negative, and it appears that the color developed with nitroprusside is due to a labile hydrogen atom in the α -position.

Studies of the hydrogenation of the angelica lactones²⁹ were of further diagnostic value. A β,γ -unsaturated lactone substituted in the γ -position (I) is hydrogenated only with cleavage of the lactone ring, giving the normal acid; but if the substituent group is located in the β -position, the double bond is saturated without cleavage of the lactone ring. Since strophanthidin can be hydrogenated without cleavage, it must contain a β -substituted, β,γ -unsaturated γ -lactone grouping, and this provides a very substantial reason for considering the lactone ring to be attached to the ring system through the β -carbon atom.

The unsaturated lactone ring is intimately associated with the physiological activity of strophanthidin and the other aglycones of the series, and it is also responsible for a remarkable, and general, isomerization. The lactone ring opens when strophanthidin is dissolved in alkali, and the product which separates on acidification is quite different from the starting material and is called isostrophanthidin.³⁰ This is a saturated substance and it no longer reacts with sodium nitroprusside. The double bond is involved and it can be shown also that the tertiary hydroxyl group at C₁₄ plays a part in the isomerization. The change has been represented as indicated in the accompanying formulas. The sub-

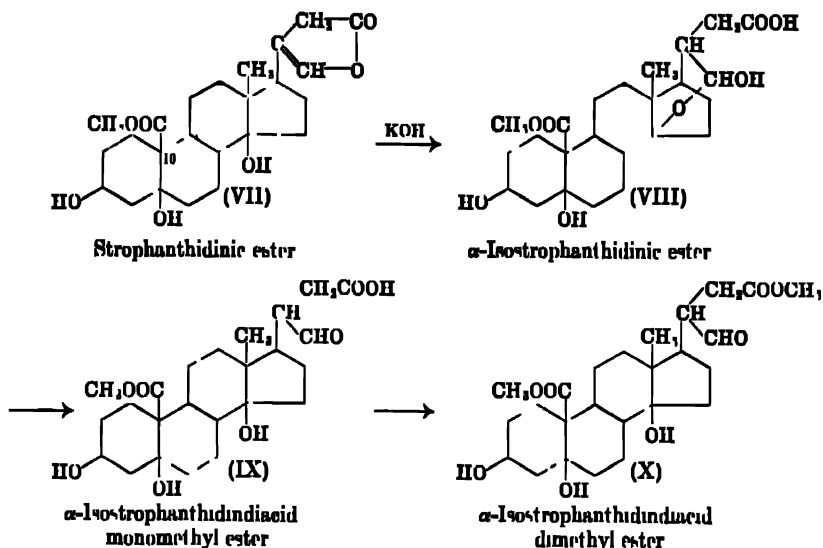


stance (a) resulting from the opening of the ring is the enol form of an aldehydic acid (b). The aldehydic group next combines with the conveniently located C₁₄-hydroxyl to produce a δ -lactol ring (c), and the acid liberated on acidifying the salt is a γ -hydroxy acid and it promptly lactonizes, giving isostrophanthidin. This lengthy sequence probably indicates all of the possible steps, but it is known that, under certain conditions, the reaction can take a simpler course. The best method of effecting the isomerization consists in treating strophanthidin with alcoholic alkali and within a short time diluting the solution with water. Most of the isostrophanthidin separates at this point and only a small amount of material is left in solution as the sodium salt (a, b, c). In this

²⁹ Jacobs and Scott, *J Biol Chem*, **87**, 401 (1920), **93**, 189 (1931)

³⁰ Jacobs and Collins, *ibid*, **61**, 327 (1924)

affecting this ester group at C₁₀, thus providing α -isostrophanthidindiacid monomethyl ester (IX), and the dimethyl ester (X) obtained on esterification forms a nicely crystalline semicarbazone. This definitely proves



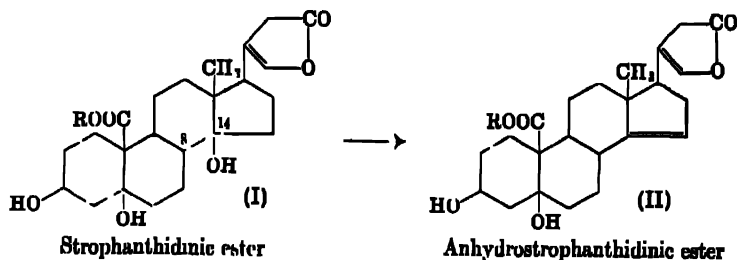
the presence of a newly formed carbonyl group, and the oxidation of X to an acid containing one additional oxygen atom (α -isostrophanthic acid dimethyl ester) shows that this is aldehydic in character.

Although the transformations which the complicated aglycones are capable of undergoing are far from simple, and although the unavoidably awkward terminology adds to the difficulty of following the changes, the evidence presented establishes conclusively the structure of the unsaturated lactone ring common to all of the aglycones of the group.

The Location of the Hydroxyl Groups. Accepting for the moment the complete establishment of the carbon skeleton of strophanthidin, there are ten possible positions in the ring system for the accommodation of the secondary hydroxyl group and five positions available for the two tertiary hydroxyl groups. A choice between the many possibilities can be made, however, without the use of reference compounds and purely by virtue of certain interrelations and interactions between the various functional groups.

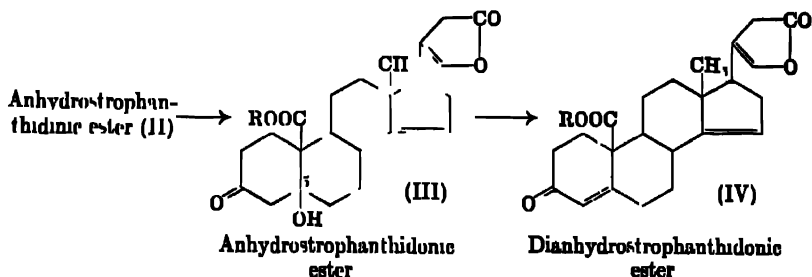
It was shown above that in the isomerization of the strophanthidin compounds by alkali a tertiary hydroxyl group enters into combination with the unsaturated lactone ring, indicating that this group is in the proximity of the lactone ring and probably at C₈ or C₁₄. It can be shown that this same hydroxyl group is the one most easily eliminated on dehy-

dration. With strophanthidin itself there is a complicating, secondary change, but the behavior of strophanthidinic ester (I) provides a clear illustration of the point.²⁴ When this is treated with alcoholic hydro-



chloric acid a monoanhydro compound (II) is easily formed. The anhydro compound, unlike I, is incapable of forming an iso-compound, from which it can be concluded that the hydroxyl group first lost is the one which ordinarily participates in the isomerization.

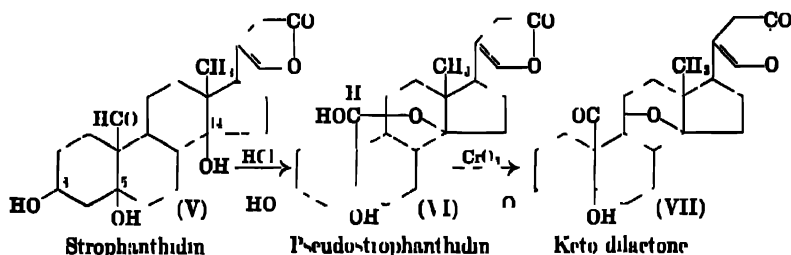
Another important relationship can be established between the secondary hydroxyl group which alone is amenable to acylation and one of the tertiary groups.²⁴ When the secondary alcoholic group is transformed to a ketonic group, as by oxidizing strophanthidinic ester to strophanthidonic ester, one of the remaining hydroxyls becomes very labile and can be eliminated by moderate heating. The labilized hydroxyl is most clearly identified by the following further changes of anhydrostrophanthidinic ester, II, described above. In this substance the hydroxyl (C_{14})



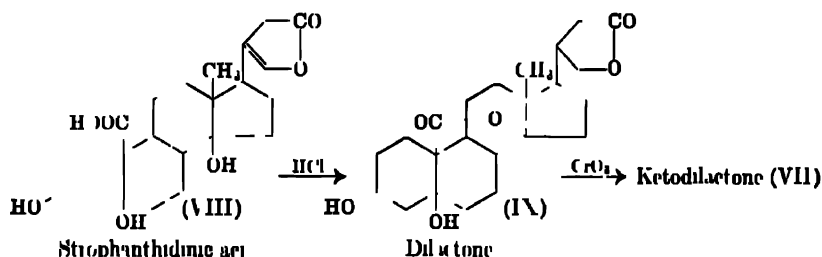
responsible for the isomerization reaction is known to be missing, but the ketone (III) obtained on oxidation has a characteristically labile hydroxyl (C_5) which is easily lost on heating. The ketone III is identified by this behavior as a β -hydroxy ketone, and it is clear that in the original aglycone there is in the β -position with respect to the secondary hydroxyl (C_8) a tertiary group (C_5) different from the one (C_{14}) associated with the unsaturated lactone ring.

The aldehydic group (C_{10}) forms a further point of reference in the molecule. By the action of concentrated hydrochloric acid, strophan-

thidin can be converted into the isomeric substance pseudostrophanthidin (VI).²³ From the fact that this forms no 140-compound it can be con-



cluded that the tertiary hydroxyl usually eliminated under the influence of mineral acids has in this instance participated in some other change. This is indeed the case, for the other two hydroxyls can be shown to be still intact. The oxidation of pseudostrophanthidin with permanganate gives a ketone²¹ (secondary OH), while chromic anhydride also attacks the lactol ring, giving a keto dilactone (VII).²³ This substance easily loses water and therefore contains a β -hydroxyl group (C₇). The third hydroxyl group (C₁₄) originally present must have combined with the aldehyde group to form a lactol ring, and it consequently is in the γ - or δ -position with respect to this group. The conclusion is confirmed by a

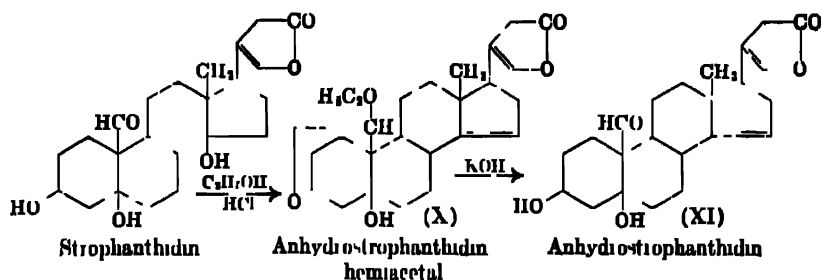


similar transformation of strophanthidinic acid (VIII→VII). This type of lactone ring is unusually stable.

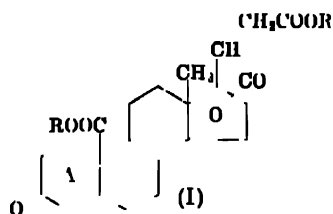
Finally, a connection is established between the secondary hydroxyl group and the aldehydic group by the conversion of strophanthidin with cold, dilute ethyl alcoholic hydrochloric acid into the hemiacetal of an anhydro compound, X.²⁴ This compound (X) forms no oxime or benzoyl derivative but the hydrolysis product (XI) is aldehydic and exhibits the characteristic test for the secondary hydroxyl group. The conclusion is inescapable that these two groups have entered into the formation of an oxide ring, and that they bear the α,γ - or the α,δ -relationship to one another.

²¹ Jacobs and Collins, *J. Biol. Chem.*, **63**, 127 (1925)

²⁴ *Idem*, *ibid.*, **59**, 713 (1924).

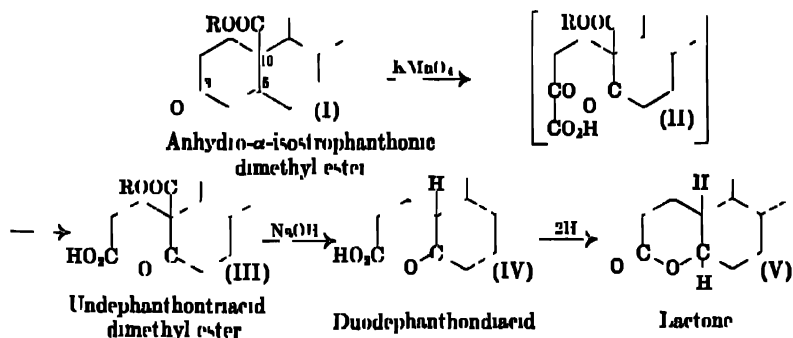


Interrelations Between Groups in Ring A. The intricate series of connections between the different functional groups reveals their relative positions in the strophanthidin molecule, and it is only necessary to establish the location of the secondary hydroxyl in the terminal ring A in order to locate them all. An elaborate and specific proof is afforded by a degradation of strophanthidin first investigated by Jacobs and Gustus.⁸⁵ The starting material was anhydro- α -isostrophanthonic dimethyl ester (I), obtained by methods similar to those given. Except for the unsatu-



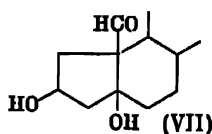
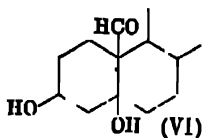
Anhydro- α -isostrophanthonic dimethyl ester

rated system in ring A, the functional groups are all modified or protected, and partial formulas will illustrate the changes. The process resembles the oxidative degradation of cholestenone (page 152). Permanganate attacks the ethylenic linkage and a keto acid (II) having one less



⁸⁵ Jacobs and Gustus, *J. Biol. Chem.*, **79**, 539 (1928)

carbon atom is formed, possibly by way of II. Undephanthontriacid dimethyl ester (III) was recognized as a β -keto ester because hydrolysis with 0.1*N* alkali resulted in decarboxylation. The ketonic group in these degradation products represents the original point of attachment of the tertiary hydroxyl (C_6) which is known to be in the β -position to the secondary hydroxyl group (C_8), and the new evidence shows that this tertiary hydroxyl is β to the original aldehydic group (C_{10}). It is known in addition that the secondary hydroxyl group is either γ or δ with respect to the aldehydic group, and there are only two arrangements which fulfill all of these conditions, namely, VI and VII:



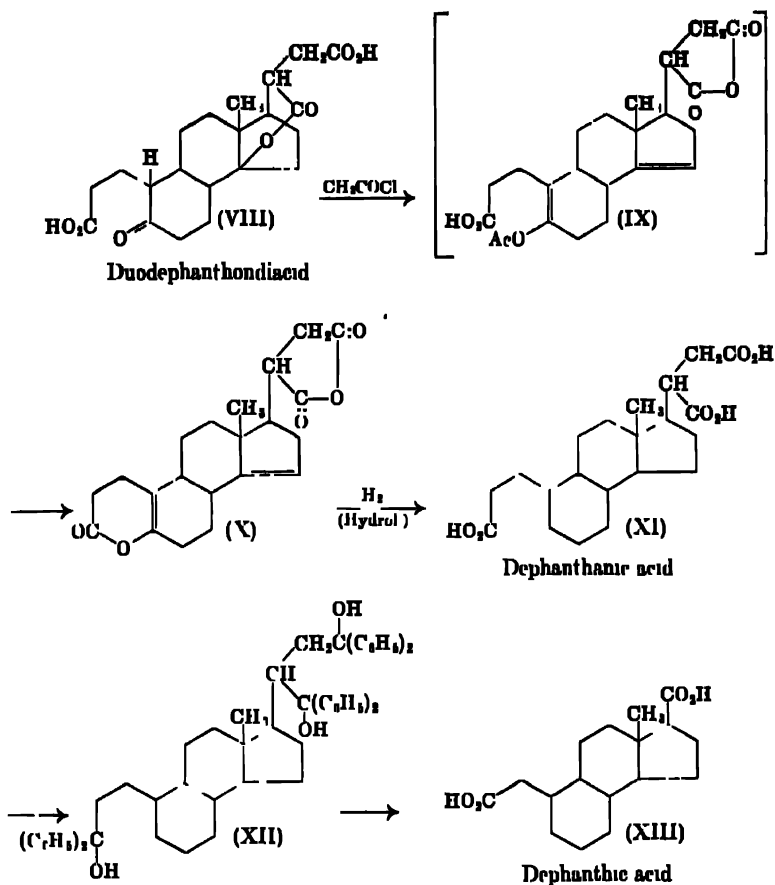
Consequently, in order to complete the evidence it is necessary to establish the size of the terminal ring. The reduction of duodephanthondiadic (IV) gives a hydroxy acid which lactonizes (δ -lactone), but while this is explained adequately on the basis of an original six-ring, the substance could equally well be a γ -lactone arising from an original five-ring. A ring of higher order than six obviously is excluded. Evidence from another source has a further bearing on the point. Working in the gitoxigenin series, Windaus³⁶ found that on opening the ring of a saturated ketone the dibasic acid produced was converted into a ketone on pyrolysis. In terms of the Blanc rule, with the validating evidence given below, this indicates an original six- or seven-ring, and only the former possibility agrees with the other evidence.

To justify the use of the Blanc rule it must be shown that the ring in question is a terminal one, and this is proved by a further degradation of duodephanthondiadic.³⁷ The reaction of this substance (VIII) with acetic anhydride and acetyl chloride³⁸ probably proceeds through an anhydro enol acetate (IX), for the product (X) is an unsaturated lactone anhydride. The lactone ring is cleaved on hydrogenation, and after hydrolysis a saturated tribasic acid (XI) results. When subjected to a Wicland degradation through the tritertiary carbinol (XII), dephanthanic acid (XI) gave a dibasic acid, dephanthic acid (XIII), having four less carbon atoms. Three of these must have come from the original lactone side chain, and the remaining one is then identified as originating from a methylene group in the original ring carrying the secondary

³⁶ Windaus, K. Westphal and Stein, *Ber.*, 61, 1817 (1928).

³⁷ Jacobs and Elderfield, *J. Biol. Chem.*, 102, 237 (1933).

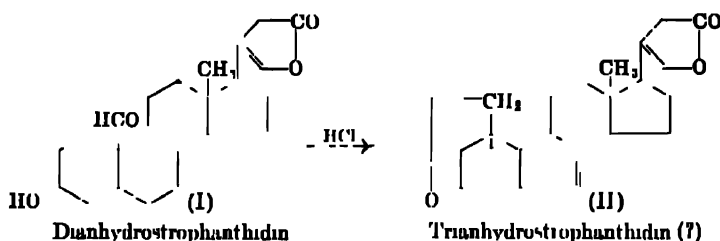
³⁸ Jacobs and Gurus, *ibid.*, 84, 183 (1929); 92, 323 (1931).



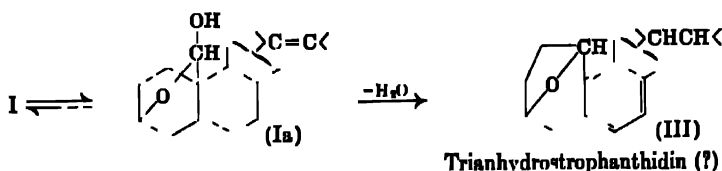
hydroxyl group The degradation as a whole indicates the changes: $-\text{CH}_2\text{COCH}_2- \longrightarrow -\text{CH}_2\text{CO}_2\text{H} \longrightarrow -\text{CO}_2\text{H}$, and the three-carbon chain can be fitted into the cholane structure only as a part of the terminal ring A.

Viewed in perspective, these degradations provide a beautiful proof of the structure of this part of the molecule, but the changes were not all correctly interpreted in the original work. Since in 1928 the similar oxidation of cholestenone was not understood, the difficulties in interpretation confronting Jacobs and Gustus are easily appreciated. It may be said that in failing to take adequate account of the loss of a carbon atom in the first step of the degradation, a great opportunity for the rapid advancement of the problem was lost. Unfortunately Jacobs and his collaborators gave more weight to a curious reaction which will be described in the following section.

Trianhydrostrophanthidin. When anhydrostrophanthidin is treated with alcoholic hydrochloric acid it is converted into the hemiacetal of dianhydrostrophanthidin, and the dianhydro compound itself is obtained on hydrolysis. On reaction with concentrated hydrochloric acid, dianhydrostrophanthidin (I) is converted into a trianhydro compound.³⁹ This differs from I in lacking the properties associated with either the carbonyl group or the secondary hydroxyl group, from which it is inferred that these have become joined together in the course of the dehydration. The reluctance of the three double bonds of trianhydrostrophanthidin to undergo hydrogenation suggested that they are present in a benzenoid ring, and this was confirmed by absorption spectra studies.³⁹ The obser-



vation that trianhydrostrophanthidin is converted on oxidation with nitric acid into mellophanic acid (benzene-1,2,3,4-tetracarboxylic acid)⁴⁰ was advanced as evidence of the presence of a benzenoid nucleus, but in view of the formation of aromatic acids from abietic acid (page 60) and ergosterol (page 172) on treatment with the same reagent, this argument probably is less valid than that based upon the absorption spectrum and the general inert character of trianhydrostrophanthidin.⁴¹ The structure of the oxidation product, however, shows that it is ring B which has been aromatized in the course of the reaction, and it is clear that the aldehydic group must have migrated from the original position at C₁₀. Because of the probable attachment to the secondary hydroxyl group, Tschesche and Knick,⁴⁰ as well as Jacobs and Elderfield,⁴¹ placed the group provisionally at C₁, in ring A, as shown in formula II. Another possibility may be suggested. The reaction perhaps proceeds through an intermediate lactol (Ia), which, because of the tertiarily bound carbinol group,



³⁹ Elderfield and Rothen, *J. Biol. Chem.*, 106, 71 (1934).

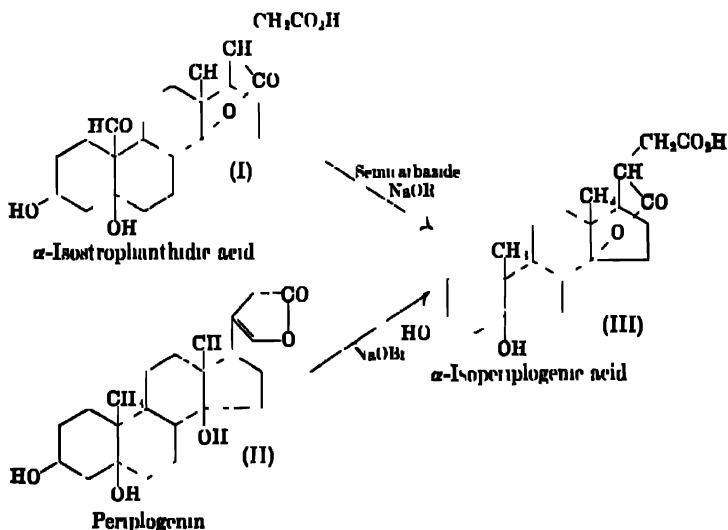
⁴⁰ Tschesche and Knick, *Z. physiol. Chem.*, 229, 233 (1934).

⁴¹ Jacobs and Elderfield, *J. Biol. Chem.*, 108, 693 (1935).

is susceptible to the Wagner-Meerwein rearrangement. Such a rearrangement would result in the enlargement of ring A by the incorporation of the original aldehydic carbon atom. According to this view trianhydrostrophanthidin may contain a seven-membered ring with a stable oxidic bridge, as in III.

Jacobs at first did not entertain the possibility of a rearrangement and he considered that the ability of one of the rings to become aromatic without loss of the aldehydic carbon atom afforded evidence that the aldehydic group does not occupy an angular position. This evidence, however, was directly contradictory to the repeated indications of steric hindrance in the reactions involving this group. The resistance to hydrolysis of the carbomethoxyl group in the corresponding position also pointed clearly to a tertiary location. The formation of similar trianhydro derivatives is not a general type of reaction but represents a unique property of strophanthidin which is not shared by the other (non-aldehydic) genins. Before discussing the events leading to the abandonment of Jacob's first interpretation, it will be well to consider the correlation of these other substances with strophanthidin.

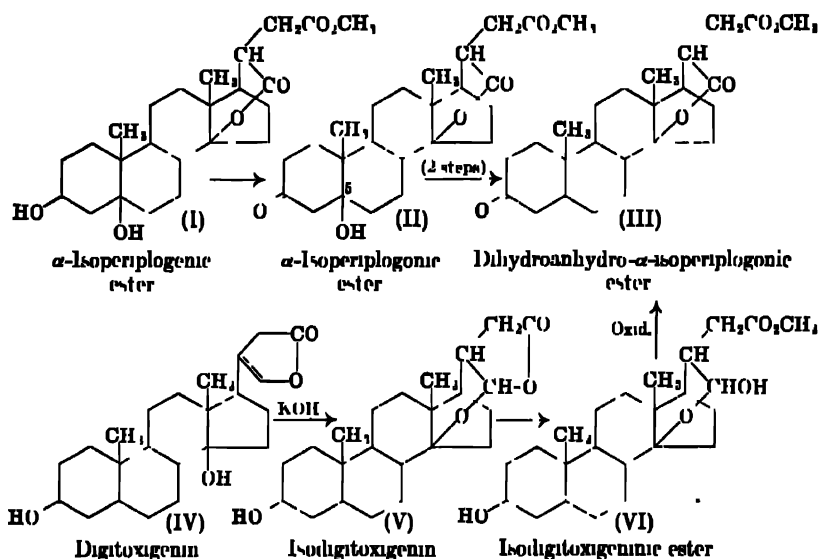
Periplogenin. In chemical behavior periplogenin is very similar to strophanthidin except that it lacks the properties associated with the presence of a free aldehydic group.⁴² Indeed the only difference in structure consists in the presence of a methyl group at C₁₀ in place of an aldehydic group. Attempts to show this by the reduction of the aldehydic group of strophanthidin met with little success because of the sensitivity



⁴² Jacobs and Hoffmann, *J. Biol. Chem.*, 79, 519 (1928).

of this substance, but it was possible to bring about the desired conversion with the use of α -isostrophanthidic acid (I), in which the latent aldehydic group of the unsaturated lactone ring has been destroyed by oxidation. The Wolff-Kishner reduction of I gave a product identical in every respect with the α -isoperiplogenenic acid (III) obtained by the hypobromite oxidation of (saponified) periplogenin (II).⁴³

Digitoxigenin. Since this genin⁴⁴ differs from periplogenin only in the absence of the tertiary hydroxyl at C₃, the two series were easily related by eliminating this group from a periplogenin compound and comparing suitable derivatives.⁴⁵ α -Isoperiplogenenic ester (I) gave the keto ester (II), which was converted through the easily formed anhydro compound (loss of the C₇-OH) to a saturated ketone (III), which was obtained in two stereoisomeric forms. Digitoxigenin (IV) was isomer-



ized (V), the lactone ring was opened and esterified, and on the simultaneous oxidation of the secondary alcoholic group and the lactol ring, a substance was obtained which proved to be identical with the higher-melting derivative III from the other series.

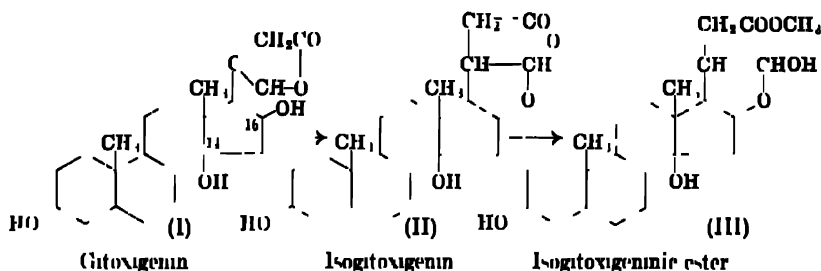
Gitoxigenin. This aglycone differs from the above members of the series in having two secondary hydroxyl groups, as indicated by the formation of a dibenzoyl derivative and a diketone. One of these groups,

⁴³ Jacobs, Elderfield, Graver and Wignall, *J. Biol. Chem.*, 91, 617 (1931); Jacobs and Elderfield, *ibid.*, 91, 626 (1931).

⁴⁴ Chemical studies: Jacobs and Gustus *ibid.*, 78, 573 (1928); Windaus and Bandter, *Ber.*, 56, 2001, (1923); Windaus and Stenn, *Ber.*, 61, 2486 (1928); S. Smith, *J. Chem. Soc.*, 2480 (1930); 1080 (1935).

⁴⁵ Jacobs and Elderfield, *J. Biol. Chem.*, 92, 313 (1931).

as will be shown presently, is located at the usual C_8 -position, while special relationships in the iso-series indicate that the second one is in the close proximity of the unsaturated lactone ring.¹⁶ The evidence in point is that the isoaglycone forms only a monobenzoyl derivative and yields a monoketone. One of the original secondary hydroxyl groups obviously has become modified in the course of the isomerization and it is inferred that this is the result of its participation in the formation of the lactol ring of the isoaglycone. The relationships are adequately explained by placing the group in question at C_{16} in ring D, as shown in formulas I-III. It is consistent with the formulation of the new, "iso"-

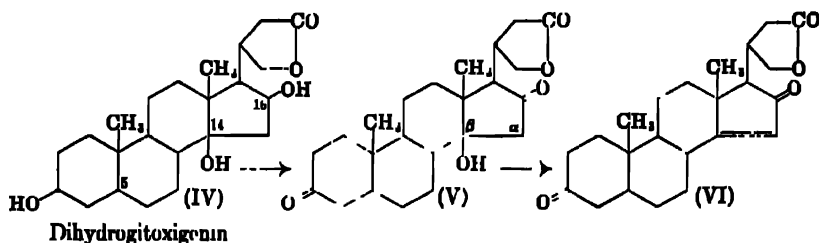


ring as five-membered that it is much more stable than the corresponding six-ring produced in the isomerization of the other aglycones through the interaction with a tertiary alcoholic group at C_{14} . When the lactone ring of isogitoxigenin (II) is opened by hydrolysis and esterification, the product (III) does not react with carbonyl reagents, indicating the absence of a hydroxyaldehyde form in equilibrium with the γ -lactol form. The corresponding compounds from the other aglycones are δ -lactols and they display aldehydic properties. It will be shown that gitoxigenin possesses a tertiary hydroxyl at C_{14} , but from the above observations it is clear that this group is less prone to enter into the isomerization than the secondary hydroxyl group at C_{16} . The difference probably is attributable to the size of the ring formed, rather than to the character of the alcoholic group.

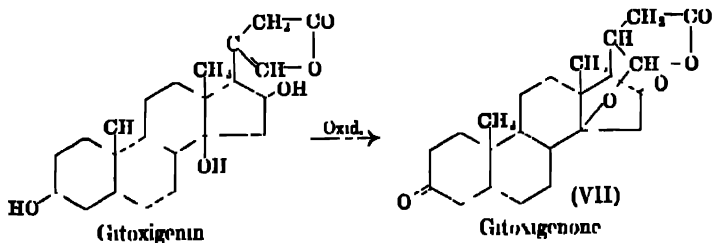
The following relationship between the two hydroxyl groups attached to ring D provides further evidence as to their location. The diketone (V) obtained by the oxidation of dihydrogitoxigenin (IV) loses the elements of water with such ease that it obviously is a β -hydroxyketone.¹⁷ If the tertiary hydroxyl is indeed in this part of the molecule, the placing of this group at C_{14} and of the secondary hydroxyl group at C_{16} is the only way of complying with this requirement and at the same time of providing for ring formation between the secondary hydroxyl group and

¹⁶ Jacobs and Gustus, *J Biol Chem*, **79**, 533 (1928), **82**, 408 (1929), **88**, 531 (1930).

¹⁷ Jacobs and Elderfield, *ibid*, **100**, 871 (1933).



the unsaturated side chain of the aglycone. For this argument to have any weight it is of course necessary to show that the tertiary group is associated with ring D, for the ready dehydration of V could be explained equally well by assuming that the hydroxyl group in question is at C₃. A proof of the point is furnished by observations having to do with an interesting isomerization which occurs in the course of the oxidation of gitoxigenin to the diketone, gitoxigenone. If this reaction involved only the dehydrogenation of the two secondary alcoholic groups, the unsaturated lactone ring should still be intact in the oxidation product, but the diketone does not respond to the Legal test and the side chain therefore is no longer unsaturated. There must be a conversion to the iso-series in the course of the oxidation, and since the secondary hydroxyl group cannot be involved, the tertiary group must participate in the reaction and therefore it is close to the point of attachment of the side chain. The only position available is at C₁₄, as in formula VII. Gitoxigenin apparently

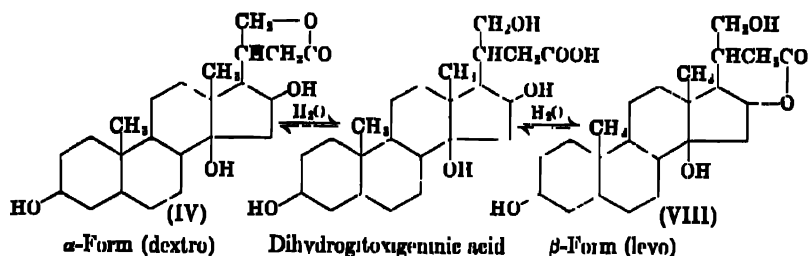


can form two different types of iso-compounds. The interaction of the secondary hydroxyl group with the side chain ordinarily takes precedence, but when this has been converted to a carbonyl group the tertiary hydroxyl enters into ready combination.

Still another kind of ring isomerism is responsible for the existence of two dihydro derivatives of gitoxigenin.^{30, 47, 48} One of these, the α -form, is represented by formula IV, above, and the structure is established both by oxidation to V and by the preparation of the compound from gitoxigenin

³⁰ Cloetta, *Arch. exp. Path. Pharmacol.*, 112, 261 (1926); Jacobs and Gustus, *J. Biol. Chem.*, 85, 531 (1930).

diacetate. The substance exhibits mutarotation, and this is due to the opening of the lactone ring and its closing in a different direction (IV→VIII). The structure of the β -form (VIII) is established by its



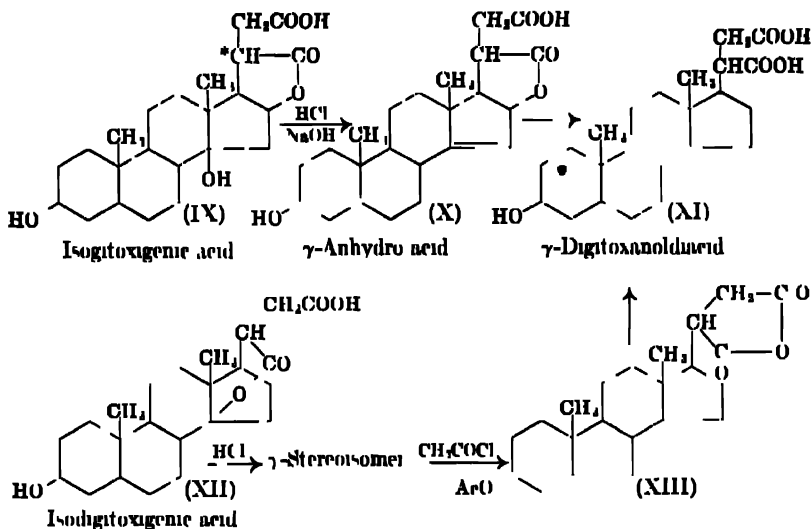
oxidation to a keto acid, $\text{C}_{22}\text{H}_{32}\text{O}_6$, the carboxyl group of which must come from a primary alcoholic group (the secondary group at C₃ is oxidized at the same time).

A close approach to a correlation of gitoxigenin with the other aglycones was made by Windaus, who converted gitoxigenin into the dianhydro compound, in which the hydroxyl group at C₃ is still intact, and converted this by hydrogenation into the hexahydro derivative.⁴⁸ A substance of the same composition was obtained from digitoxigenin⁴⁹ by similar methods. The tetrahydroanhydrodigitoxigenin, however, was not identical with the hexahydrodianhydrogitoxigenin but isomeric with this substance. The difference is maintained in the two ketones obtained on oxidation, and in the desoxylactones obtained by reduction of the ketones according to Clemmensen, and it probably is of a stereochemical nature. The saturation of the multiple double bonds in the anhydro compounds offers ample opportunity for isomerism in ring D and in the side chain.

The problem finally was solved by Jacobs and Gustus,⁵⁰ who succeeded in obtaining identical derivatives from the two genins by taking advantage of an isomerization to a new (γ) stereochemical series under the influence of strong hydrochloric acid. Isogitoxigenic acid (IX) was converted through the C₁₄-chloro compound into an anhydro derivative (X). Possibly an inversion at C₂₆ occurs in the course of the reactions, for the chloro compound on hydrolysis yields a stereoisomeride of IX. When the ester of X was submitted to hydrogenation the lactone linkage was cleaved simultaneously with the saturation of the double bond, giving the dibasic acid XI. This was identical with the corresponding acid of the digitoxigen series which was obtained from XII by isomerization, conversion to the acetyl anhydroanhydride (XIII), hydrogenation, and hydrolysis. This proved conclusively that gitoxigenin has the same car-

⁴⁸ Windaus and Freese, *Ber.*, **58**, 2503 (1925)

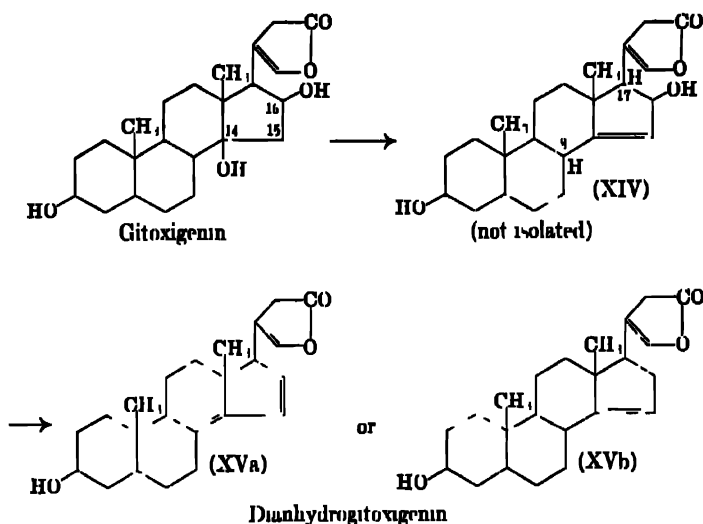
⁵⁰ Jacobs and Gustus, *J. Biol. Chem.*, **86**, 199 (1930).



bou skeleton as the other aglycones and that it contains a similarly situated secondary hydroxyl group.

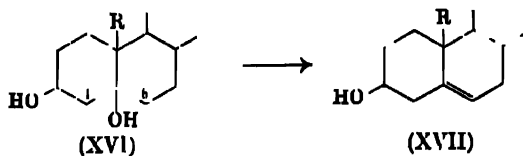
There is one special point in the chemistry of gitoxigenin which is of considerable interest in connection with certain observations which will be presented below. Although the aglycone contains only one tertiary hydroxyl group, it yields a dianhydro compound when treated with cold, concentrated hydrochloric acid (Windaus and Schwarte⁸). One of the two secondary hydroxyl groups evidently is eliminated with great ease, and the group in question can be identified as the one located in the five-membered ring. In none of the other series thus far discussed is there any instance of a secondary hydroxyl group possessing the lability displayed in the present case, and the unique behavior must be due to some special feature of structure. The double bond in the lactone ring plays no part of importance, for dihydrogitoxigenin, in either the α - or the β -form, yields a dianhydro derivative (Windaus, Westphal and Stein¹⁰).

A consideration of the probable course of the unusual reaction suggests an explanation. In analogy with all known cases it is reasonable to suppose that the tertiary group at C₁₄ is first eliminated, giving XIV. The double bond established at C₁₄-C₁₇ is in a position to activate the secondary hydroxyl group in ring D, and the presence of an allylic system would account well for the lability of the group in question. The loss of the second molecule of water may involve the hydrogen atom at either of the tertiary locations C₈ or C₁₇. A 1,4-elimination would give XVa, while a 1,2-elimination would lead to the somewhat less likely structure XVb. The dianhydro compound XVa might also arise through an allylic



shift of the hydroxyl group to C₁₄, and dehydration, but such an explanation is unnecessary. Whatever the mechanism, the driving force derived from the tendency to assume a conjugated system accounts adequately for the reaction.

According to this view the ready dehydration of the secondary alcoholic group of gitoxigenin is associated with the presence of a tertiary hydroxyl group in the β -position, but the 1:3 disposition of the two substituents merely provides the opportunity for the establishment of a conjugated system and does not necessitate a double dehydration. With strophanthidin or periplogenin, for example, there are no indications that the secondary hydroxyl group in the structure XVI is subject to elimination following the removal of the tertiary group in the β -position (as in the formation of dianhydrostrophanthidin). A likely interpretation of the difference is that the initial loss of water involves the 6- rather than



the 4-position, giving a substance (XVII) in which the double bond is too far removed from the hydroxyl group to influence it. This view is supported by the fact that the 5,6-position is a favored location for the double bond in all of the unsaturated alcohols of the sterol and sex hormone series.

Reactions somewhat analogous to that assumed to occur in the second step of the dehydration of gitoxigenin have been observed in the morphine series. In the acetolysis of α -methylmorphinethine (page 26), for example, a secondary hydroxyl group is eliminated from a position adjacent to a double bond. A still closer parallel is afforded by a recent observation of Evans and Schoenheimer⁵¹ These investigators prepared

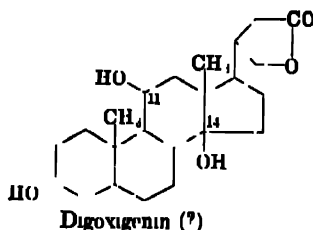


epiallocholesterol (XVIII), a new isomer of cholesterol, by the reduction of cholestenone with aluminum isopropylate. They found that the substance is distinguished from other sterols by being quantitatively dehydrated by brief treatment with dilute alcoholic hydrochloric acid solution at the boiling point, with the formation of the hydrocarbon XIX or a bond isomer. The loss of water occurs readily in this case even though one feature of possible importance in the gitoxigenin structure is lacking. The hydrogen atom eliminated from epiallocholesterol comes from a methylene group, whereas in the case of monoanhydrogitoxigenin (XIV) the hydrogen is eliminated from a tertiary location at C₁₇ or at the bridge head C₅. There are certain indications that the presence of a lone hydrogen atom in an available position favors the establishment of an unsaturated linkage, one being that cholic acid can be converted under rather moderate conditions of dehydration into 3,12-dihydroxy- Δ^7 -cholenic acid (page 132). Of the three secondary hydroxyl groups only that at C₇ is adjacent to a bridge head carrying a hydrogen atom, and consequently it is significant that it is this group which is preferentially eliminated and that the double bond established extends to the bridge head. Another indication in the same direction is the behavior of the bromination products of derivatives of aetiocholanone-3 and aetioallocholanone-3 (page 250.) Whereas the 2-bromo compounds lose hydrogen bromide with great difficulty, a bromine atom at C₄ easily separates with a hydrogen atom from the bridge head C₅ to establish an ethylenic linkage.

It is evident that the lability of a hydroxyl group does not of necessity establish its tertiary character, for a secondary hydroxyl group is also labile when it is adjacent to a double linkage or when it is present in the combination: $>C(OH)CH_2CH(OH)CH<$.

Digoxigenin. This aglycone, characterized extensively by Smith,⁵² has not been correlated with the other members of the group but it closely parallels these compounds in properties and reactions (Legal test, conversion to digoxigeninic acid, conversion to an iso-compound.) The resemblance to digitoxigenin is particularly striking, for the only differences are those occasioned by the presence of an additional secondary hydroxyl group. The aglycone is isomeric with gitoxigenin, and like this substance it contains one tertiary hydroxyl group (located at C₁₄ by the iso-reaction) and two secondary groups. It yields a diketone (digoxigenone) on oxidation and, in contrast to gitoxigenin, the unsaturated lactone ring is not disturbed in the process, since digoxigenone can be isomerized to a compound identical with that resulting from the oxidation of isodigoxigenin. One of the carbonyl groups apparently occupies a somewhat hindered position, for Smith was able to obtain only a monoxime and a monosemicarbazone from digoxigenone, isodigoxigenone, or dihydrodigoxigenone. The lack of reactivity appears to be only relative, however, for a dioxime was obtained from anhydrodigoxigenone. In the formation of anhydrodigoxigenin only one molecule of water is eliminated, and consequently a secondary hydroxyl group does not appear to be in close association with the tertiary group as in the case of gitoxigenin.

Smith notes that in all probability digoxigenin has the carbon skeleton common to the other aglycones of the group. The experimental evidence



locates the tertiary hydroxyl group (C₁₄) and the double bond. One of the secondary groups probably is at the favored 3-position (Smith), and it is suggested that the most probable location for the other one is at C₁₁. A group at this position, which is sufficiently remote from both the side chain and the tertiary hydroxyl group to account for the lack of interaction, either direct or indirect, should be subject to some steric hindrance.

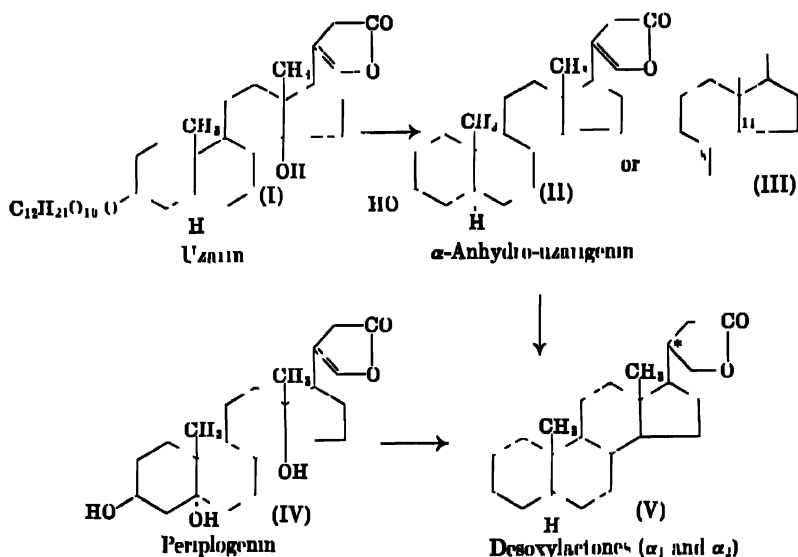
Uzargenin. In a preliminary investigation Windaus and Haack⁵³ found that under the drastic conditions required for the hydrolysis of the glucoside uzarin the genin is converted into an anhydro compound. Continuing this work at the Göttingen Laboratory, Tschesche⁵⁴ isolated in

⁵² S. Smith, *J. Chem. Soc.*, 1305 (1935), and earlier papers.

⁵³ Windaus and Haack, *Ber.*, 63, 1377 (1930).

⁵⁴ Tschesche, *Z. physiol. Chem.*, 222, 50 (1933).

addition to the main product, α -anhydro-uzarigenin (m.p. 265°), a small amount of a β -anhydro compound (m.p. 237°) which presumably differs only in the location of a nuclear double bond, although evidence on this point is lacking. Tschesche at first regarded the substances as dianhydro compounds, but concluded⁵³ from more recent analyses and hydrogenation experiments that they contain but one double linkage in addition to that recognized as being in the side chain (Legal test). The presence of an original hydroxyl group at C₁₄ would account for the elimination of water in two directions, as indicated in Tschesche's provisional formulas II and III, and more specific evidence of the location of the group in question is found in the fact that uzarin can be converted into an iso-compound with



alcoholic alkali.⁵⁵ Since the sugar residue is not lost in the process it must be attached to the secondary hydroxyl group recognizable in the anhydro compounds by their ability to form monoacetyl derivatives.

Tschesche achieved a correlation⁵⁴ with periplogenin as follows. α -Anhydro-uzarigenin gave on hydrogenation (as the acetate) a mixture of two tetrahydro derivatives (α_1 and α_2) which were later shown to differ in the configuration at the new center of asymmetry in the side chain. The two isomers were converted by oxidation to the ketones and these were reduced by the Clemmensen method to saturated desoxylactones of the formula V. The α_2 -desoxylactone proved to be identical with an octahydrotrianhydroperiplogenin which Jacobs and Bigelow⁵⁶ had

⁵³ Tschesche and Rohle, *Ber.*, **68**, 2252 (1935)

⁵⁶ Jacobs and Bigelow, *J. Biol. Chem.*, **101**, 897 (1933)

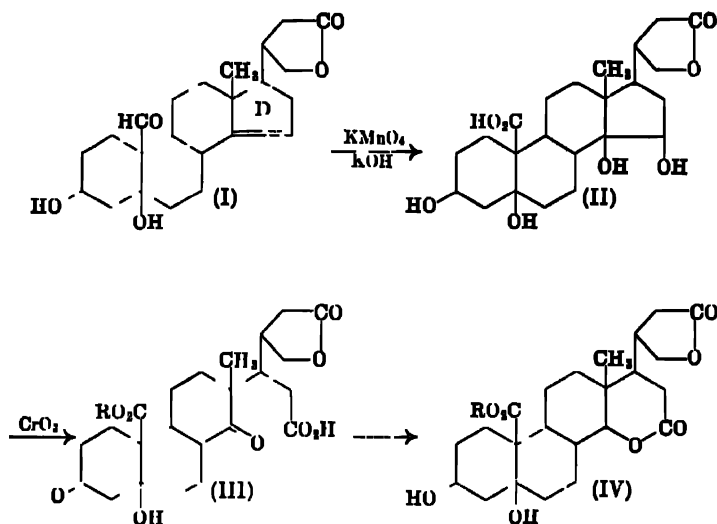
obtained from periplogenin (IV). This establishes the identity of the skeletal structures of the two aglycones, but it should be noted that the observation does not prove that these substances correspond in the configuration of the ring system. Since periplogenin carries a hydroxyl group at C₃, an inversion can occur at this point in the course of the hydrogenation of an anhydro linkage. There is no opportunity for such an inversion in the reactions of uzarin, and evidence which will be presented below establishes the configuration at C₃ as that of the members of the cholestane series (A/B:trans)

Tschesche⁵⁴ converted the two tetrahydroanhydro-uzarigenins through the ketones into dibasic acids of the bilanic acid type and found that both acids yield ketones on pyrolysis. This indicates that the secondary hydroxyl group is contained in the terminal, six-membered ring A of the cholane system. It was later observed⁵⁵ that α -anhydro-uzarigenin is precipitated by digitonin, and in view of the specificity of the reagent Tschesche considers that the hydroxyl group very probably is located at the usual 3-position. The configuration at this center evidently is that of dihydrocholesterol and coprosterol. It was found that sterol derivatives having a hydroxyl group at C₄ (cholestanol-4) are not precipitated by digitonin

In comparison tests the striking observation was made that other cardiac aglycones are probably *epi*-compounds, as they fail to form stable digitonides. Neither digitoxigenin, gitoxigenin, digoxigenin, strophanthidin, nor anhydrosarmentogenin is precipitated by digitonin. It is very interesting that uzarin, which appears to differ from the other plant heart poisons in the spatial arrangement of the secondary hydroxyl group at C₃, possesses at most very feeble cardiotonic properties. The lack of pronounced physiological activity may be due in part to this divergence from the usual stereochemical pattern, but there is evidence that uzarin also differs from other glycosides of the group in the arrangement of rings A and B, and this may also be a determining factor.

The Early Conception of the Ring System. It was shown above that the degradation of strophanthidin to duodephanthondiacid and dephanthic acid furnished reliable evidence that the ring system common to all of the aglycones includes a terminal six-membered ring carrying a secondary hydroxyl group. The presence of a terminal five-ring was established by Jacobs and Elderfield⁵⁷ in a degradation involving the opening of this ring (D), as follows. On oxidizing anhydrodihydrostrophanthidin (I) with permanganate in acetone solution only the aldehydic group is attacked, but when the oxidation is conducted in the presence of

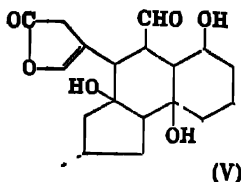
⁵⁷ Jacobs and Elderfield, *J. Biol. Chem.*, **97**, 727 (1932). A similar degradation of gitoxigenin has been reported by the same authors, *ibid.*, **96**, 367 (1932).



alkali the substance also is attacked at the double bond, giving a glycol acid (II). On oxidation of the ester of II with chromic anhydride the glycol linkage is cleaved with the opening of ring D and the production of a diketo acid III, the secondary alcoholic group being simultaneously oxidized. The hydroxy acid obtained on reduction of the two carbonyl groups readily lactonizes to IV. Since this stability is characteristic of a δ -lactone (or possibly a γ -lactone) but not of lactones of a higher order, the original ring cannot contain six or more carbon atoms and very probably is a five-ring. It may be added that it is easily shown that in the lactone IV the original hydroxyl groups at C₃ and C₇ are still intact, because on the conversion of the former to a carbonyl group, the latter becomes mobile.

One other inference regarding the ring system was deduced (incorrectly) from the work on trianhydrostrophanthidin. Because the aldehydic residue in this substance was thought to be attached to the ring which had become aromatized, it was considered that this was the original location and hence that the original ring was a six-ring. Since the nucleus carrying the aldehydic group was regarded as different from that containing the secondary hydroxyl group, the presence in strophanthidin of two six-membered rings was considered established.

From a consideration of the facts available at the time, Jacobs²⁷ in 1933 advanced tentatively the partial formula V for strophanthidin. The state of the problem resembled in many ways the condition of the sterol-bile acid work at the time of the early Wieland-Windaus formulation (1928). In each case a number of interconversions and reactions had



established the nature of the functional groups and certain relationships between them. The different members of each of the two broad groups of natural products had been for the most part adequately correlated, and the side chains were completely characterized. The German workers had attempted to determine the nature of the sterol-bile acid ring system by numerous partial oxidative degradations at various parts of the molecule, but they had been misled by the failure of the Blanc rule in certain specific cases, and one serious error of interpretation by Wieland had insinuated itself into the reasoning and remained undetected. Jacobs had followed a similar program of oxidative degradation and he also had been misguided partly through the unforeseen molecular rearrangement which occurs in the formation of trianhydrostrophanthidin, and partly because of failure to take adequate account of the first step in the degradation to duodephanthonic acid and of the hindered character of the (tertiary) aldehydic and carboxylic groups at C₁₀. It will be recalled that Ruzicka, in his investigations of abietic acid, had similarly been led to disregard evidence pointing to the presence of a tertiarily bound carboxyl group because of the results of a dehydration reaction which was recognized later as involving a molecular rearrangement.

A solution of the sterol-bile acid problem finally was indicated by the introduction of a new experimental method which supplied information regarding the ring system as a whole, and clearly in 1933 similar information concerning the cardiac aglycones was sorely needed for the proper consolidation of the great wealth of data regarding the many transformations of these versatile substances. The selenium method of Diels, which had been of such service in the one field, was soon found to be an invaluable guide in directing the other series of investigations into a course which ultimately led to a solution.

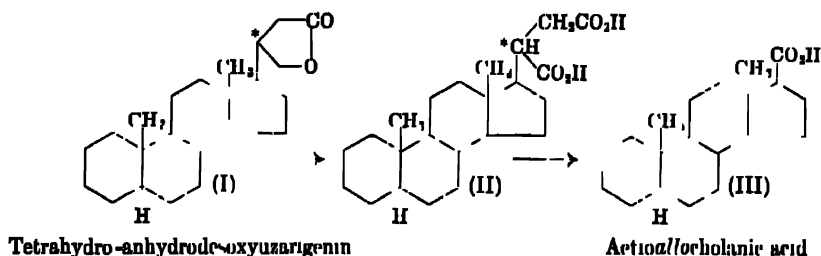
Dehydrogenation of the Aglycones. Jacobs and Fleck⁵⁸ investigated the dehydrogenation of strophanthidin in 1931 and obtained a substance closely resembling the Diels hydrocarbon (3'-methyl-1,2-cyclopentenophenanthrene) in composition and melting point and giving no depression when mixed with a sample of the material. Their product, however, yielded a quinone on oxidation, whereas all the investigators who have

⁵⁸ Jacobs and Fleck, *Science*, **73**, 133 (1931); *J. Biol. Chem.*, **97**, 57 (1932).

studied the oxidation of the Diels hydrocarbon have reported negative results. The substance was regarded at the time as 1,2-dimethylphenanthrene, but later work has shown that this was incorrect and that the material probably was not a single individual. The next publication on the subject was by Tschesche and Knick,⁵⁰ who succeeded in obtaining from the dehydrogenation of 30 g. of anhydro-uzarigenin 0.1-2 of a hydrocarbon, m.p. 124-125°, which was fully identified as the Diels compound. Shortly afterwards, Elderfield and Jacobs⁵⁰ reported the isolation of the Diels hydrocarbon from the mixture obtained by the action of selenium on strophanthidin at a more carefully controlled, and probably a lower, temperature (320-340°) than in the experiment of Jacobs and Fleck. The supposed dimethylphenanthrene was not encountered again; at a higher temperature they obtained an unidentified hydrocarbon, $C_{21}H_{16}$, m.p. 295-297°.

The independent isolation in two laboratories of a well-established degradation product of the sterols and bile acids was most suggestive, and once the hypothesis of a cholane ring system for the cardiac aglycones had in this way become firmly established, it became a matter of the greatest interest to submit this conception of the structure to rigorous appraisal. In view of the drastic nature of the pyrolytic treatment with selenium and considering that five carbon atoms are lost in the process, the isolation of the Diels hydrocarbon constituted a valuable suggestion rather than a proof of the ring system.

Establishment of the Carbon Skeleton. Following his work on the dehydrogenation, Tschesche⁵¹ carried out a further degradation of the two stereoisomeric desoxylactones (α_1 and α_2) previously obtained from



α -anhydro-uzarigenin (page 284). On oxidation, each lactone (I) was converted into a dibasic acid (II), the isomerism due to the asymmetric center (*C) being preserved. Each acid, in the form of the diester, was submitted to the Wieland degradation, that is, it was converted into the

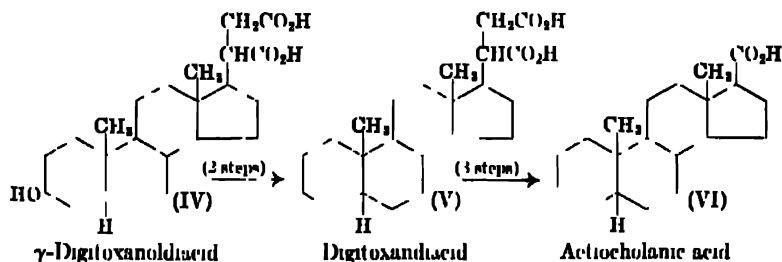
⁵⁰ Tschesche and Knick, *Z. physiol. Chem.*, **222**, 58 (1933).

⁵¹ Elderfield and Jacobs, *Science*, **79**, 279 (1934); *J. Biol. Chem.*, **107**, 143 (1934).

⁵² Tschesche, *Z. angew. Chem.*, **47**, 720 (1934); *Z. physiol. Chem.*, **220**, 219 (1934).

tetraphenyl ditertiary carbinol and the latter was oxidized. The center of asymmetry being destroyed, both isomers gave the same monobasic acid (III), the substance having the composition of Wieland's actiocholanolic acid (page 146). The degradation product, however, was not identical with Wieland's acid, and the stereoisomer of the *allo*-series was at the time unknown. A degradation of III through the Grignard derivative gave a dibasic acid isomeric with Wieland's aetiobilianic acid and behaving in a similar manner on pyrolysis, which strengthened the belief that the difference is only a stereochemical one. Tschesche prepared a small sample of actioallocholanolic acid and made a preliminary identification with the degradation product, and he later⁶² prepared a quantity of the former substance and fully established the identity of III with this compound. The reference acid was prepared from hyodesoxycholic acid through allocholanolic acid,⁶³ nor-, and bismor-allocholanolic acids, the latter substances having recently become available through the work of Chuang⁶⁴ and Fernholz.⁶⁵

Shortly after the appearance of Tschesche's first paper, Jacobs and Elderfield⁶⁶ announced a very similar degradation in the digitoxigenin series. Starting with γ -digitoxanoldiacid, IV (page 280), the saturated dibasic acid V was obtained by the Clemmensen reduction of the corresponding ketone. Subjected to the Wieland degradation, this gave a product (VI) identical with Wieland's actiocholanolic acid.



These two important investigations prove conclusively that the cardiac aglycones of the strophanthus-digitalis-uzara group have the reduced cyclopentenophenanthrene ring system characteristic of the sterols, bile acids, and sex hormones, and that the disposition of the twenty-three carbon atoms is precisely that of the corresponding part of the sterol structure. The side chain of the aglycones is shorter by one carbon atom than that of the bile acids, but it has the same branched structure. As for the

⁶² Tschesche, *Ber.*, 68, 7 (1935).

⁶³ Windaus and Bohne, *Ann.*, 433, 278 (1923).

⁶⁴ Chuang, *ibid.*, 500, 270 (1933).

⁶⁵ Fernholz, *ibid.*, 507, 128 (1933).

⁶⁶ Jacobs and Elderfield, *Science*, 80, 434 (1934); *J. Biol. Chem.*, 108, 497 (1935).

configuration of the rings, the isolation of stereoisomeric aetiocholanolic acids in the two degradations is a matter of significance because there is in neither series of transformations any opportunity for the disturbance of the original configurations at C₃ in the aglycones. Uzarigenin and digitoxigenin belong to the cholestane (trans) and the coprostane (cis) series, respectively. Since gitoxigenin has been correlated with digitoxigenin by reactions offering no possibility of inversion at C₆, this aglycone must be a cis compound. The correlation of periplogenin with digitoxigenin proves that the aglycone has the same skeletal structure as the other compounds, but since the transformations include the hydrogenation of a C₅-unsaturated anhydro derivative of periplogenin the configuration of the end product affords no indication of the original spatial arrangement of the aglycone. The reactions correlating periplogenin and strophanthidin offer no chance for inversion at C₅, and consequently these substances belong to the same, but as yet undetermined, stereochemical series. The development of the complete stereochemistry of the aglycones and of their many transformation products is largely a problem for the future, although some progress has been made already in interpreting the various phenomena in stereochemical terms.^{66, 67}

Ouabain. This glycoside is treated separately from the others for the reason that the evidence of structure is very incomplete and because both the genin and its simple derivatives are unknown.

In 1888, Arnaud⁶⁸ isolated the active principle extracted by water from the bark and root of the ouabaio tree and long used by the East African Somalis as an arrow poison. He recognized the substance as a glycoside and called it ouabain. The ouabaio wood itself, a quantity of which was secured by an explorer, was found to yield as much as 3 g. of the glycoside per kilogram. Later, Arnaud⁶⁹ found the same glycoside in the seeds of *Strophanthus gratus*, the inéc (or onaye) used by the Pahouins as an arrow poison. The active principle, which is one of the most toxic of the cardiac glycosides, is called both ouabain and g-strophanthin. The substance crystallizes unusually well and it can be obtained in a completely pure form more easily than the other poisonous principles. For this reason ouabain is widely employed as a standard in the assay of commercial digitalis and strophanthus preparations. The substance is very rare and it is used but little as a drug.

Arnaud⁷⁰ identified the sugar residue as rhamnose, but he was unable to obtain the genin, the material being resinified under the drastic con-

⁶⁶ Elderfield, *Chemical Reviews*, 17, 187 (1935).

⁶⁷ Arnaud, *Compt. rend.*, 106, 1011 (1888).

⁶⁸ *Idem*, *ibid.*, 107, 1182 (1888).

⁶⁹ *Idem*, *ibid.*, 126, 346, 1208 (1898)

ditions required for hydrolysis. By the action of sodium ethylate in alcoholic solution, the glycoside was converted (by hydrolysis of the lactone ring) into a crystalline salt without rupture of the glycosidic linkage. The corresponding acid, ouabaic acid, was also isolated.⁷¹ Ouabain crystallizes with nine molecules of water of crystallization and the preparation of a pure anhydrous sample suitable for analysis presents difficulties. A number of derivatives were analyzed, however, and the results were interpreted by Arnaud as pointing to the formula $C_{30}H_{48}O_{12}$. Many years later, Jacobs and Bigelow⁷² pointed out that the analytical figures agree even better with the formula $C_{20}H_{44}O_{12}$, and their revised formulation was well grounded by new analyses and by the characterization of a number of additional derivatives. In this more recent work it was shown that ouabain, unlike its dihydro derivative, gives the characteristic Legal reaction and therefore probably contains the usual unsaturated lactone ring of the other members of the group.²⁷ The glycoside was also found to yield an iso-compound under the influence of alcoholic potassium hydroxide, and isouabain gave a negative Legal test. In analogy with the other isomerizations, this is good evidence of the location of a tertiary hydroxyl group at C_{14} .

The other reactions of ouabain presented more perplexing problems. Arnaud⁷³ succeeded in converting the glycoside by means of acetic anhydride and zinc chloride into the heptaacetyl derivative of anhydroouabain. Jacobs and Bigelow established for the compound the formula $C_{43}H_{56}O_{18}$ and pointed out that the tertiary hydroxyl group (C_{14}) responsible for the iso-reaction must be the one lost in the formation of the anhydro linkage. Three acetyl groups must be located in the sugar residue, and it can be inferred that four hydroxyl groups of the genin moiety have been acetylated. The heptaacetyl compound absorbed two moles of hydrogen on hydrogenation and on submitting the saturated heptaacetyl compound to acetolysis (acetic and hydrochloric acids), Jacobs and Bigelow were able to isolate a sugar-free compound which was characterized by the preparation of derivatives as an acetoxylactone, $C_{24}H_{30}O_4$. The remarkable feature of the reaction was the loss of one of the original carbon atoms of the genin moiety as formaldehyde. Arnaud⁷⁴ had reported a similar loss of carbon in a careful study of the action of nitric acid on ouabain in which he isolated sugar-free nitro and dinitro compounds. Jacobs and Bigelow suggested that in their acetolysis reaction the formaldehyde arises from the cleavage of the group $:C=CH_2$. The

⁷¹ Arnaud, *Compt. rend.*, 126, 1280 (1898).

⁷² Jacobs and Bigelow, *J. Biol. Chem.*, 96, 847 (1932).

⁷³ Arnaud, *Compt. rend.*, 126, 1654 (1898).

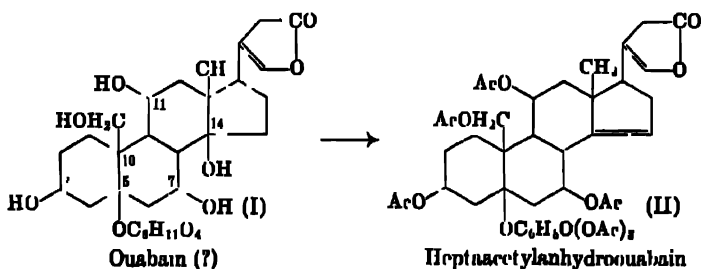
⁷⁴ *Idem*, *ibid.*, 126, 1873 (1898).

acetoxylactone has but one (modified) hydroxyl group, while the compound from which it is formed has five such groups. One of these holds the sugar residue and the others are acetylated. No less than four modified hydroxyl groups are eliminated in the acetolysis, and Jacobs and Bigelow considered that they must, in consequence, occupy tertiary positions.

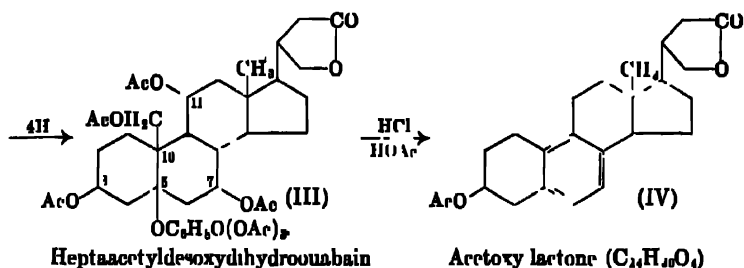
There are certain inconsistencies in this interpretation and, in view of the inaccessibility of the poison and its importance as a standard of cardiotonic activity, an alternate, if admittedly speculative, view may be presented.

Kon¹⁵ has observed that it is difficult to see how the doubly bound methylene group suggested by Jacobs and Bigelow can be accommodated to the skeleton common to the other cardiac glycosides. It is also inconsistent with general observations in other series that the four alcoholic groups of ouabain which are capable of being acetylated are all tertiary. A fifth group, eliminated in the acetylation, doubtless is tertiary (C_{11}) in character, but the fact that the others can be acetylated indicates, by the best analogy, that they are either primary or secondary. The group holding the sugar residue may well occupy a tertiary position, as at C_7 . Since this group and three of the acetyltable groups are eliminated in the acetolysis they must be intimately associated in the molecule, for example in β -positions to one another. Such an arrangement requires that there be no more than one secondary hydroxyl associated with each of the rings A, B, and C and the conclusion is reached that the fourth group capable of undergoing acetylation is primary in character, that is, that one of the angular methyl groups is hydroxylated.

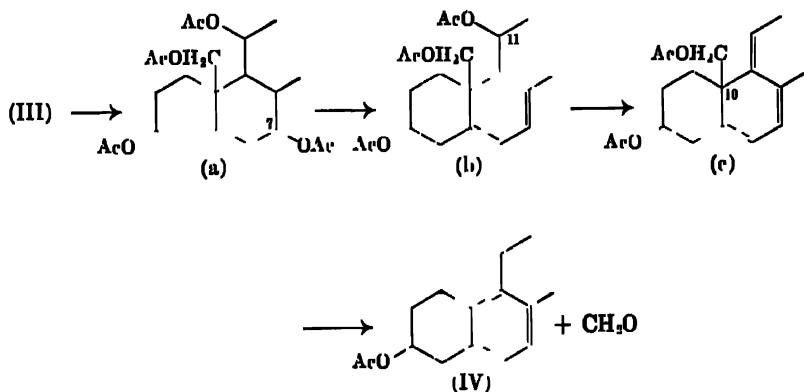
A possible arrangement which appears to satisfy all the conditions is shown in formula I. The groups available for acetylation are those at C_4 , C_7 , C_{11} (secondary), and the $-\text{CH}_2\text{OH}$ group at C_{10} , and the formation and hydrogenation of the heptaacetyl compound may be represented as in formulas II and III. Formula IV is suggested as a possible struc-



¹⁵ Kon, *Chem. Soc. Annual Reports*, 31, 237 (1935)

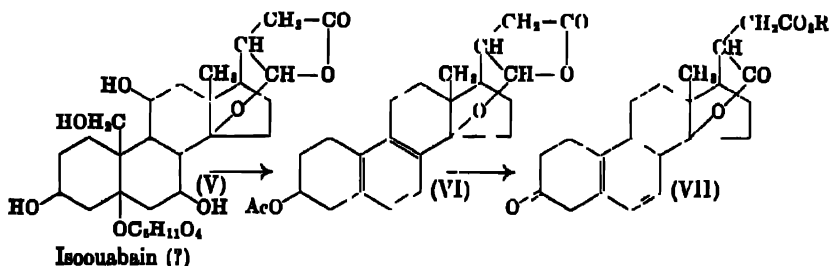


ture for the product of acetolysis, and it is supposed that the driving force for the reaction comes from the tendency of ring B, with its cluster of oxygen-containing groups, to assume the aromatic structure. In a saturated substance of the structure of III the tertiary group at C_5 probably would be the most vulnerable point of attack, and the double bond produced when it is eliminated (a) would labilize the group in the β -position C_7 (see page 280). The cleavage of this group would give a sub-



stance (b) having a conjugated system, and the conjugation could be extended, in turn, by the elimination of the acetoxy group at C_{11} , giving (c). In this phase (c) there would be two special features of structure, namely, the close approach of ring B to the aromatic condition, and the tertiary bound, primary carbinol group at C_{10} . The latter structure represents a type susceptible to the Wagner-Meerwein rearrangement, and it is not difficult to imagine that some variety of molecular shift, coupled with the disposition of the three double bonds to seek the same nucleus and form a stable benzenoid ring, could lead to the expulsion of formaldehyde and the formation of IV. It will be recalled that the elimination of a tertiary bound carbon residue under similar conditions has been observed frequently in the morphine series, for example, in the acetolysis of α -methylnorphimethine (page 26).

In another series of experiments Jacobs and Bigelow⁷⁶ treated isouabain (V) with acetic anhydride and sulfuric acid and obtained a monoacetyl trianhydrolactone. According to the above interpretation, this substance is assigned the structure VI. The lactone ring of the



desacetyl compound from VI can be opened by hydrolysis and the acid group esterified. The ester on oxidation gives a keto lactone (VII). Clearly the whole isoglycone grouping comprising the two oxygen bridges is still intact and one of the original secondary hydroxyl groups likewise survives the anhydro reaction. The location of this group in the terminal ring at a position removed from a bridge head (in contrast to C_{11}) would account for its failure to participate in the succession of dehydrations.

The suggested interpretation of the acetolysis reactions assumes the presence of an aromatic ring in each of the two cleavage products, but the experimental evidence is not decisive on this important point. Jacobs and Bigelow state that the acetoxy lactone (IV) from heptaacetyldeoxydihydroouabain absorbs three moles of hydrogen to give a mixture of two saturated stereoisomerides, the desacetyl derivatives of which yield ketones on oxidation. While the availability of the three double bonds for hydrogenation clearly argues against the view that they are present in a single, aromatic nucleus, another observation of Jacobs and Bigelow offers some support for the above structures. The desacetyl compound from the product (VI) obtained from isouabain was found to be remarkably resistant to hydrogenation under conditions permitting the ready absorption of hydrogen by less highly unsaturated compounds. The substance was wholly unattacked in neutral solvents, although it was found possible to achieve a hydrogenation in glacial acetic acid solution with the absorption of three moles of hydrogen. The behavior of the compound was said to be reminiscent of that of trianhydrostrophanthidin. In addition to these observations there are some indications that Arnaud's⁷⁴ nitro nor-derivatives (possibly $\text{C}_{22}\text{H}_{25}\text{O}_6(\text{NO}_2)$ and $\text{C}_{22}\text{H}_{25}\text{O}_6(\text{NO}_2)_2$) contain an aromatic ring. The compounds may arise from the dehydrating and

⁷⁶ Jacobs and Bigelow, *J. Biol. Chem.*, 101, 15 (1933)

oxidizing action of nitric acid on ouabain, followed by nitration. The hypothesis that the various products of degradation contain a benzenoid ring appears at least sufficiently plausible to warrant further experimental inquiry.

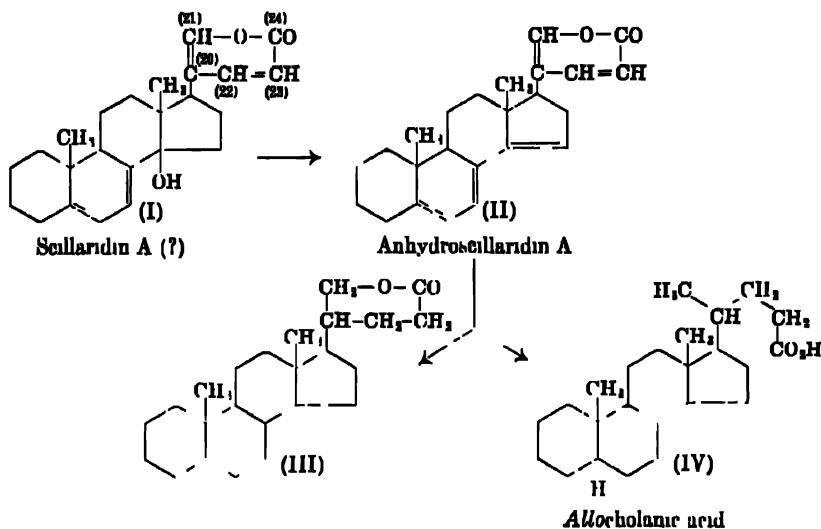
Scillaridin A. Although scillaren A, one of the glycosides from squills, closely resembles the other heart poisons in physiological action, the aglycone scillaridin A differs markedly in certain respects from the aglycones of the *strophanthus-digitalis-uzara* group. It contains one more carbon atom than these compounds, it fails to give the Legal nitroprusside reaction, there are four double bonds in the molecule, and there are differences in the course of the isomerization by alkali. Stoll and his collaborators, after a ten-year period of investigation at the Sandoz laboratory, published in 1933 the first of a series of papers on the subject of scillaren A and its aglycone.⁷⁷ Numerous careful analyses of scillaridin A, its derivatives and transformation products, at first pointed to the formula $C_{27}H_{42}O_3$ for the aglycone, but early in 1935 an observation was made which cast serious doubt on this apparently well-established formulation. In a three-step process, involving no drastic conditions or any reactions likely to disturb the carbon skeleton, Stoll obtained a saturated monobasic acid which proved to be identical with *allocholanic acid*.⁷⁸ The bile acids are regarded as C_{24} -compounds, although the evidence is based more on degradation experiments than on direct elementary analysis. Ordinary combustions are hardly sufficient to distinguish between compounds of such high molecular weight. For scillaridin A, for example, the differences in carbon and hydrogen content for the formulas $C_{27}H_{42}O_3$ and $C_{24}H_{36}O_3$ are only 0.26% and 0.23%, respectively. Stoll later⁷⁹ found it possible to determine the molecular weights of the cholanolic acids with an accuracy of 2-3 units by the titration of sufficiently large samples, and the results dispelled his previous doubts that *allocholanic acid* and scillaridin A are C_{24} -compounds.

The conversion to *allocholanic acid* proves conclusively that the carbon skeleton of the aglycone is precisely that of the bile acids. On this basis, and from such other observations as have been made, Stoll has suggested for scillaridin A the provisional formula I. The degradation was accomplished through the anhydro compound II, which can be prepared by the high-vacuum sublimation of either the aglycone or the glycoside, or by warming scillaridin A with alcohol and concentrated hydrochloric acid. On hydrogenation in glacial acetic acid solution with

⁷⁷ Stoll and collaborators, *Helv. Chim. Acta*, 16, 703 (1933); *Z. physiol. Chem.*, 222, 24 (1933); *Helv. Chim. Acta*, 17, 641, 1334 (1934), 18, 32, 401, 644 (1935).

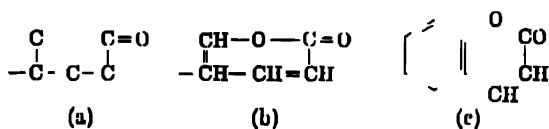
⁷⁸ Stoll, A. Hofmann and Helfenstein, *ibid.*, 18, 644 (1935).

⁷⁹ Stoll, A. Hofmann and Peyer, *ibid.*, 18, 1247 (1935).

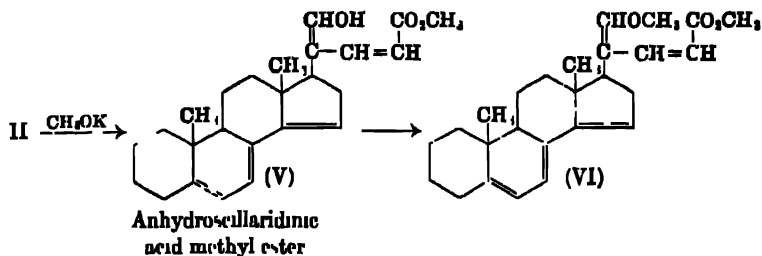


Adams' catalyst, anhydroseallaridin A gave a mixture of the saturated lactone III and the desoxy acid IV. These substances are formed simultaneously, and undoubtedly the lactone ring is cleaved while it is still in the unsaturated condition. The acidic product of hydrogenation was at first assigned the formula $C_{27}H_{42}O_2$, but on further investigation two stereoisomeric acids were isolated in a pure condition and one of them (α -scillanic acid) proved to be identical with *allocoholanic acid*.

As for the lactone ring of the aglycone, the degradation establishes the skeleton (a), and the double unsaturation, as in (b), seems necessary

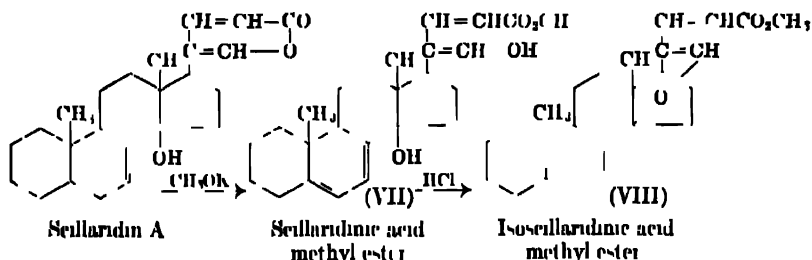


to account for the close analogy with coumarin (c) disclosed in several experimental comparisons. Since (b) does not represent a β,γ -unsaturated lactone, the formulation is consistent with the failure to respond to the Legal test. It is consistent with the six-ring structure that the lactone ring opens easily and tends to stay open. This is shown best in the case of anhydroseallaridin A (II), which (because of the absence of the C_{14} -hydroxyl group) is incapable of forming an iso-compound. Methyl alcoholic potassium hydroxide cleaves the lactone ring of II with simultaneous esterification of the carboxyl group, giving (V). This substance appears to be entirely enolic, for it is strongly acidic (titration), it gives acyl derivatives, and it fails to form an oxime, semicarbazone, or phenyl-



hydrazone. This indicates that the substance has little tendency to exist in the form of the δ -aldehyde unsaturated ester. Another indication of the enolic character of the ester is the ability of the substance to form the ether, VI, on reaction with methyl alcohol and hydrogen chloride. On hydrolysis of anhydroseallaridinic ester (V) with aqueous alkali, followed by acidification, the original anhydroseallaridin A is regenerated.

Seallaridin A itself reacts with methyl alcoholic potassium hydroxide in an exactly analogous manner to form an enolic ester similar to V, but on recrystallization the compound easily loses water and it has not been obtained in a pure condition. The freshly prepared substance is converted by diazomethane into an ether-ester analogous to VI, and under hydrolytic conditions the ester linkage is cleaved without affecting the ether group. According to Stoll the loss of water from seallaridinic acid methyl ester (VII) is due to the formation of an iso-compound (VIII).

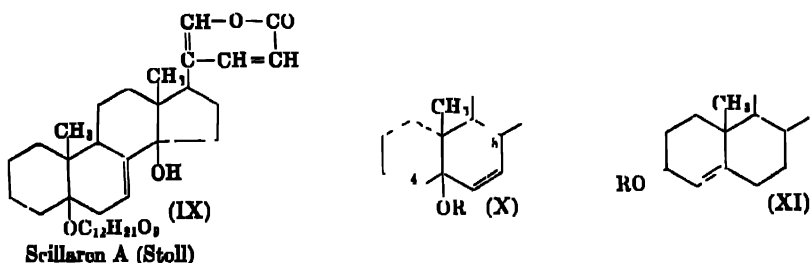


The oxide ring of the isoaglycone is considerably more stable to hydrolysis than the corresponding five-membered oxide ring of the *strophanthus-digitalis* isoaglycones.

The hydroxyl group concerned in the formation of the isoaglycone evidently is tertiary in character, for seallaridin A does not yield acyl derivatives and it is easily converted into an anhydro compound. The hydroxyl group must be located in the neighborhood of the lactone ring to account for the formation of the isoaglycone, and the stability of the oxide bridge in this compound can be accounted for only by placing the hydroxyl group at C₁₄, allowing the formation of a six-membered oxide ring. It

can be inferred that in the original glycoside the C_{14} -hydroxyl group is not bound to a sugar residue, for scillaren A can be converted by means of alcoholic alkali through the enolic ester to an ester of the iso-series without cleavage of the biose residue.

In his provisional formula for scillaridin A Stoll placed the nuclear double bonds at the 5,6- and 7,8-positions partly because, like ergosterol, the aglycone gives a characteristic Rosenheim color reaction with trichloroacetic acid. Furthermore, it seems necessary to indicate a conjugation between the ethylenic linkages in order to account for a special relationship between scillaridin A and its glycoside. Although the biose residue (rhamnose-glucose) of scillaren A is of the type which ordinarily is very resistant to hydrolysis, the glycoside can be converted quantitatively into scillaridin A by the action of 1% sulfuric acid in 50% methanol solution at the temperature of the steam bath. The sugar unit (R) apparently is eliminated by cleavage (of HOR) rather than by hydrolysis, for a new double bond appears in the genin moiety as a result of the reaction. Stoll interpreted the lability of the hydroxyl group holding the sugar residue as an indication that it is in a position such that it is activated by an ethylenic linkage of the glycoside, and this view was confirmed by the observation that this lability vanishes on saturation of the linkage in question with hydrogen. All attempts to hydrolyze hexahydroscillaren A were unsuccessful. Stoll suggested formula IX for scillaren A as indicating this relationship, but in analogy with other cases (page 280) it would be expected that a 7,8-double bond would labilize the tertiary hydroxyl group at C_{14} rather than the modified group at C_5 . It is more likely that the ethylenic linkage is in the α,β -position with respect



to the carbon atom holding the biose unit, as in X. A substance having the bond structure attributed by Stoll to scillaridin A might be produced from X by the 1,4-elimination of HOR across the ring. The formula for scillaridin A is open to some question, however, for if the aglycone contains a conjugated system extending from C_6 to C_9 it is difficult to see why the C_{14} -hydroxyl group is not more labile. The elimination of this group and the extension of the conjugated system into ring D would be

expected to occur under the conditions employed for the hydrolysis of scillaren A. It is conceivable, if less likely, that a substance of the structure X might lose HOR from the 4,5-position, and unsaturation at this point would account equally well for the positive Rosenheim reaction shown by the aglycone. In view of the recent observation that the secondary hydroxyl group of epiallocholesterol is subject to ready elimination (page 282), it seems possible that scillaren A has the structure of XI, and this also would account for the color test and for the apparent absence in scillaridin A of an ethylenic linkage in the α,β -position to the C₁₄-hydroxyl group, for the conjugated system would extend from C₂ to C₃. It is odd, however, that although the ready formation of scillaridin A from the glycoside indicates that the new double bond assumes a position of conjugation, there is no appreciable difference in the absorption spectra of scillaridin A and scillaren A. Both substances show an absorption maximum at 300 m μ , which possibly is due to the system of two double bonds conjugated with the unsaturated oxygen atom in the lactone ring. An effect due to changes in the nucleus as the result of the hydrolysis is not apparent. Clearly the problem of structure requires further investigation.

Physiological Activity of the Glycosides and Aglycones.⁸⁰ The plant heart poisons exert such a powerful and specific action on the cardiac muscle that bio-assays of these substances can be performed rapidly and with a considerable degree of precision. The frog method⁸¹ is one of the most widely used procedures for the evaluation of the digitaloid drugs, and it is particularly well adapted to water-soluble products. Ouabain, probably the most potent glycoside of the group, is taken as the standard of activity. The minimal systolic dose of ouabain is between 0.00046-0.00054 mg. per gram of frog's body weight. The activity is also expressed in terms of frog doses per milligram of material: approximately 2000 F. D. per mg. of ouabain. The limiting toxic dose per gram varies somewhat from animal to animal but not over a very wide range. For comparing the activity of digitaloid drugs and for estimating the therapeutic dose the Hatcher cat method⁸² has certain special advantages. The drug is always administered by intravenous injection. The poison is very much less toxic when taken by mouth.

Chen, Chen and Anderson⁸³ made a careful comparison of several crystalline cardiac principles by the Hatcher cat method with the results

⁸⁰ Gley, *Compt rend.*, 107, 349 (1898); Straub, *Biochem Z.*, 75, 132 (1918); Jacobs and Hoffmann, *J. Biol. Chem.*, 74, 787 (1927); S. Smith, *J. Chem. Soc.*, 508 (1930); Stoll and co-workers, *Helv. Chim. Acta*, 16, 703 (1933); 13, 401 (1935); Cloetta, *Arch. exp. Path. Pharmacol.*, 112, 261 (1926); Windaus, Rohrer and Schweizer, *Ber.*, 57, 1386 (1924); K. K. Chen and A. L. Chen, *Arch. intern. pharmacodynam.*, 47, 297 (1934); Gessner, *Arch. exp. Path. Pharmacol.*, 148, 351 (1930).

⁸¹ The Pharmacopoeia of the United States of America, 10th Edition, 394 (1926).

⁸² Hatcher and Brody, *Am. J. Pharm.*, 32, 360 (1910).

⁸³ K. K. Chen, A. L. Chen and R. C. Anderson, in press.

given in the table. These investigators report that gitoxin was not sufficiently soluble to permit standardization, and that uzarin exhibited no digitalis-like action. According to Gessner uzarin has about one-sixtieth the activity of ouabain. The nature of the sugar residue appears to be

Drug	Cat unit in mg per kg
Ouabain (g-Strophanthin)	0.12±0.002
Cymarín	0.13±0.003
Scillaren A	0.15±0.007
Digoxin	0.22±0.008
Digitoxin	0.33±0.008
Theriatin ¹⁴	0.92±0.035

of little importance, for different glycosides of the same genus are very similar in activity. *g*-Strophanthin and cymarín, for example, are practically equivalent, as are digitanide A and digitoxin. While itself not toxic, the sugar portion of the molecule influences the character and intensity of the cardiac effect, probably by virtue of its influence on the water-solubility and the diffusibility of the material. The toxicity invariably decreases on the removal of the sugar moiety, and the cardiotonic effect of the aglycone usually is less persistent than in the case of the glycoside. The degree of activity of an aglycone appears to be quite dependent upon its solubility in water. Strophanthin is fairly soluble in water and it approaches the glycoside in toxicity (one-third as toxic), scillarenin A is very sparingly soluble in water and it is about one-tenth as active as scillarenin A.

The number of free hydroxyl groups in the genus part of the molecule varies from one (scillarenin A) to five (ouabain), and there are instances of the occurrence of these groups in all of the four rings. In all but possibly one case a secondary hydroxyl group is located at the characteristic 3-position, as in the steroids. It was noted in an earlier section that uzarin differs from most of the other glycosides in the spatial arrangements at C₁ and C₂ (β -type, *allo-series*). It is possible that the almost complete absence of cardiotonic properties in uzarin is connected with these stereochemical differences, for the substance is closely related in structure to the more potent glycosides, for example, to digitoxin. Anhydrodigitoxigenin probably differs from α - or β -anhydro-uzarigenin only in having the *epi*-configuration at C₁, and a *cis* linkage between rings A and B, and yet its glycoside is characterized by a high degree of potency. It will be recalled that in the case of androstene the physiological activity drops to one-seventh the original value following an inversion at C₃, while an inversion at C₇ results in the complete loss of activity. It would be of great interest to determine separately the effects of epimerization and allomerization in

¹⁴ A glycoside from the still units. See K. K. Chen and A. L. Chen, *J. Pharmacol.*, **49**, 561 (1933); **51**, 23 (1934); *J. Biol. Chem.*, **105**, 231 (1934); Chatsak, *Chem. Abs.*, **27**, 5171 (1933); **29**, 7011 (1935).

the present case, and to inquire further into a possible parallelism in the relationships of structure and configuration to the two kinds of physiological actions. As a matter of pure speculation it seems likely that the arrangement of the C_3 -hydroxyl group is the more important factor in determining the activity of the heart poisons, as in the precipitability of sterols by digitonin. It may be a matter of significance that of all of the aglycones investigated only the one from the weakly cardiotonic uzarin is precipitated by digitonin. Possibly a C_3 -hydroxyl group in the β -arrangement normal to the sterols interferes in some way with the cardiac effect, as by promoting the formation of an inactive complex with constituents of the blood stream. The *epi*-arrangement may offer no more opportunity for such inactivation than if the hydroxyl group were absent, which possibly is the case with scillaren A, one of the most active of the poisons. From the present evidence there is little reason to believe that the number and positions of the other hydroxyl groups are of great importance except, perhaps, as they influence the solubility.

There are definite indications that the cardiac effect is intimately associated with the unsaturated lactone ring. With the substances of the digitalis-strophanthus group the saturation of the ethylenic linkage in the lactone ring results in a great decrease in toxicity. Dihydroouabain and dihydrocymaridin are approximately one sixteenth and one twenty-third as active as the original glycosides (Jacobs). Other dihydro compounds have been reported to be completely inactive (Windaus, Cloetta). Another variety of unsaturated lactone ring is present in scillaren A, but it apparently has an entirely similar function. The saturation of the ethylenic linkages in this case practically wipes out the toxic character of the substance (Stoll). The conversion of a glycoside into an iso-compound also destroys the original condition of unsaturation in the lactone ring, and it is significant that the iso-compounds are completely inactive. When the lactone ring is opened without hydrogenation and without hydrolysis of the sugar grouping, the activity may not be entirely lost but it at least drops to the order of one five-hundredth of the original value (Straub, Stoll). From this it appears that the original condition of the lactone ring is very important. The fact that a latent aldehydic group having reducing properties is liberated on hydrolysis of the lactone ring in the case of the drugs of the digitalis-strophanthus group would be taken as a clue to the mechanism of the cardiac effect, were it not that in the case of scillaren A the corresponding group is entirely enolic and devoid of reducing properties. The mechanism is still entirely obscure.

It would be of considerable interest to know something of the rôle of the aetiocholan ring system. The complicated ring structure may serve simply as a convenient frame for the support of the effective lactone

grouping and for the attachment of solubilizing groups. It does not appear impossible that this question can be answered by the investigation of synthetic substances having similar lactone groups attached to different ring systems.

TOAD POISONS

That there is present in the toad an active, poisonous principle has been recognized since antiquity, and although the nature of the poison was endowed with various legendary beliefs throughout the middle ages it has long been recognized that the venom of the toad has definite medicinal qualities. For centuries the Chinese have employed as a drug a dried preparation from a common toad. The remedy is known as Ch'an Su in China and as Senso in Japan. Ch'an Su is sold in the form of hard, dark brown cakes which are applied externally in the treatment of toothache, sinusitis, and hemorrhages of the gums. Dried and powdered toad skins were commonly used as a remedy for dropsy until Withering introduced the use of the foxglove drug. It has been known for nearly a century that the poison of the toad has a specific, digitalis-like action on the heart, the intravenous injection of very small doses in frogs promptly inducing systolic standstill.

The poison is located in skin glands and material for experimental purposes or for use as a medicinal can be obtained either from dried toad skins or from the living animal. The bulk of the poisonous secretion is contained in glands located behind the eyes and it may be obtained by expression. The toad suffers no ill effects from the treatment, and regeneration of the so-called "parotid" glands takes place after the removal of the secretion. Investigation has failed to reveal any use made by the toad of its own poison, either in self-defense or in body functions, and the rôle of the venom in the animal organism is no more clear than is the function of the physiologically active alkaloids and heart poisons of plants.⁸⁵

Early chemical studies of the constituents of toad poisons were undertaken by Faust, and by Phisalix and Bertrand. The American workers Abel and Macht⁸⁶ (1911) were the first to accomplish the isolation of an active principle in a pure condition. The substance was named bufagin (L. *bufo*, toad). The exploration of the chemistry of the toad poisons has been conducted principally by H. Wieland, at the Freiburg and Munich laboratories. Pharmacological and chemical studies have been conducted in this country by H. Jensen, of the Johns Hopkins University, in collaboration with K. K. Chen, of the Lilly Research Laboratories.

The skin secretions of toads have been found to contain any or all of the following classes of compounds: bufotoxins (conjugated genins),

⁸⁵ K. K. Chen and A. I. Chen, *Arch. intern. pharmacodyn.*, **47**, 207 (1934).

⁸⁶ J. J. Abel and Macht, *J. Amer. Med. Assoc.*, **56**, 1531 (1911), *J. Pharmacol.*, **3**, 319 (1911).

bufagins (genins), sterols, adrenaline, and bufotenines. The bufotoxins and the bufagins are responsible for the cardiotoxic activity of the secretions, and they will form the chief subject for discussion below. The chief constituent of the sterol fraction is cholesterol, admixed, in some instances, with ergosterol.⁸⁷ Adrenaline has not always been detected in the poisonous fluid, but there are instances of its occurrence in astonishingly large amounts. Abel and Macht⁸⁶ isolated the material in crystalline form, along with bufagin, from the poison of the large South American toad, *Bufo agua* (more properly called *B. marinus*), the concentration being approximately 5 per cent of the secretion. Adrenaline also has been obtained from Ch'an Su⁸⁸ and from the secretion of the tropical toad, *B. arenarum*,⁸⁹ and its presence in other species of toads has been established by color tests and by means of blood-pressure measurements on pithed rats (Chen, Jensen and Chen⁸⁷). It is surprising that the active principle of the suprarenal glands should occur also in the skin glands of the toad, and the amount of material stored in the "parotid" glands of a single Jamaican toad (*Bufo marinus*) is more than four times the amount present in a pair of human suprarenal glands.⁸⁵ It is interesting that the Chinese drug Ch'an Su has been found to contain, in addition to the cardiotoxic agents, this pressor substance of recognized astringent and hemostatic properties.

The presence in the secretions of alkaloid-like organic bases other than adrenaline was indicated by the work of Phisalix and Bertrand,⁹⁰ who obtained in a crude form a basic substance to which they gave the name bufotenine. Handovsky⁹¹ prepared several crystalline salts of the compound, and the formula $C_{14}H_{18}O_2N_2$ was fully established by Wieland in 1931.⁹² Wieland obtained the substance from the common European toad *Bufo vulgaris* and he isolated from the same source a second substance, bufotenidine, which proved to be the quaternary ammonium base of bufotenine. Bufotenidine was also isolated by Wieland from Ch'an Su, and Jensen and Chen⁸⁵ found in this poison a substance probably identical with bufotenine.⁹³

⁸⁷ K. K. Chen, Jensen and A. L. Chen, *Proc. Soc. Exptl. Biol. Med.*, **20**, 905, 907 (1932), *ibid. J. Physiol.*, **97**, 511 (1931), 101, 20 (1932), *J. Pharmacol.*, **49**, 1, 11, 26 (1933); K. K. Chen and A. L. Chen *ibid.*, **49**, 503, 514, 526 (1933).

⁸⁸ Jensen and K. K. Chen, *J. Biol. Chem.*, **82**, 397 (1924), **87**, 741 (1930).

⁸⁹ Deulofeu, *Z. physiol. Chem.*, **237**, 171 (1935).

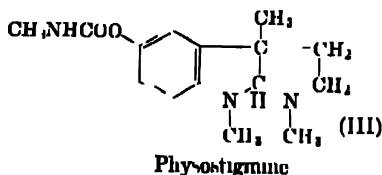
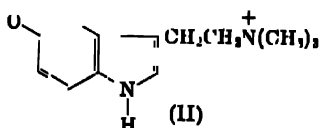
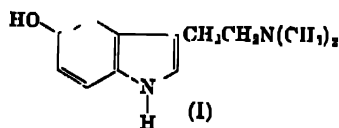
⁹⁰ Phisalix and Bertrand, *Compt. rend. soc. biol.*, **45**, 477 (1893).

⁹¹ Handovsky, *Arch. exptl. Path. Pharmacol.*, **86**, 135 (1920).

⁹² Wieland, Hesse and Mittasch, *Ber.*, **64**, 2098 (1931).

⁹³ Jensen and K. K. Chen, *ibid.*, **65**, 1310 (1932), investigated the skin secretions of twelve species of toads from five different continents and separated from the basic fractions crystalline "bufotenines" as salts, mostly as flavanates. Without demonstrating the purity or the individuality of these preparations, each was given a name composed of a prefix, indicating the name of the species or the place of origin, and the generic term "bufotenine". While exhibiting some differences in pharmacological activity and in composition, these materials were all very similar, and the existence of pure bases other than Wieland's bufotenine and bufotenidine has yet to be demonstrated.

On the grounds of certain pharmacological observations, Jensen and Chen⁹³ expressed the conjecture that bufotenine is a derivative of tryptamine, and this proved to be a correct inference. In later work, Wieland⁹⁴ was able to establish by synthesis the structure of bufotenine (I) and its betain derivative (II). Bufotenine bears a striking structural

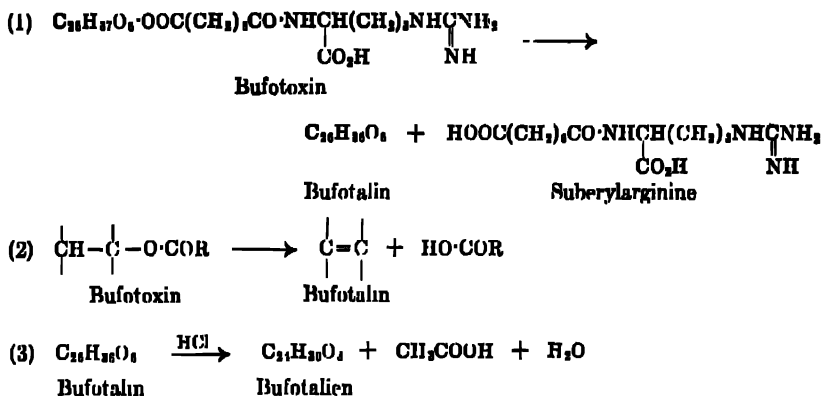


relationship to physostigmine (III), the principal alkaloid of the Calabar bean. Although many naturally occurring substances are known to be derived from 6-hydroxyindole, and probably are synthesized in the cell from tyrosine, bufotenine and physostigmine are the only known natural derivatives of 5-hydroxyindole, and their origin is still obscure. Bufotenine has a pressor action similar to that of adrenaline but less pronounced. It produces a rise in blood pressure in pithed cats due to both vasoconstriction and cardiac stimulation.

The Cardiotoxic Constituents. The active poisons of the toad secretions occur in the form of conjugated compounds which occupy a position roughly corresponding to that of the cardiac glycosides, for on hydrolysis of a conjugated compound, or bufotoxin, a genin (bufagin) is liberated. The best-known example is the compound bufotoxin, from *Bufo vulgaris*, discovered and characterized by Wieland and Alles.⁹⁵ This substance ($C_{40}H_{62}O_{11}N_4$, m.p. 205°) is the suberylarginine ester of the genin bufotalin. In the animal organism bufotoxin apparently undergoes cleavage to a certain extent according to Equation 1, as detailed in Equation 2. The process is not one of hydrolysis but involves the elimination of the conjugated acid with the production of a double linkage in the genin moiety. Under truly hydrolytic conditions the conjugated acid is converted into suberic acid and arginine. Under such conditions bufotalin loses one molecule each of acetic acid and water and yields bufotalien, as in (3). Bufotalin is thus the acetyl derivative of a com-

⁹³ Wieland, Koss and Mittsach, *Ann*, **513**, 1 (1934), see also Hoshino and Shimodaira, *ibid.*, **520**, 10 (1935).

⁹⁴ Wieland and Alles, *Ber*, **55**, 1789 (1922).



pound which is easily converted into a dianhydro derivative, and in consequence of this instability the hydrolysis of bufotoxin by dilute acids yields only bufotalien, along with suberylarginine (or its hydrolysis products). Since toad secretions are found to contain both bufotoxin and bufotalin in varying proportions, it is inferred that in the organism the cleavage takes the course defined in the first equation.

Wieland and Voëke⁹⁸ isolated a similar conjugated compound from the dried skins of Japanese toads and called the substance gamabufotoxin, "gama" being the Japanese word for toad. Gamabufotoxin ($\text{C}_{34}\text{H}_{50}\text{O}_{10}\text{N}_4$, m.p. 210°) differs in composition from bufotoxin only in the absence of an acetyl group, and on acid hydrolysis it yields suberic acid, arginine, and a substance, m.p. 204° ($\text{C}_{24}\text{H}_{42}\text{O}_4$), which is not the true genin, but which can be obtained from it. The genin itself, gamabufogenin, was found in the toad secretion along with gamabufotoxin. On treatment with concentrated hydrochloric acid it is converted into an anhydro compound, m.p. 261° , having the same composition as the hydrolysis product of gamabufotoxin, but it is not identical with this substance (m.p. 204°). On heating the hydrolysis product with concentrated hydrochloric acid, however, it rearranges to anhydrogamabufogenin, m.p. 261° .

The conjugated compounds and the genins are similar in physiological activity, the suberylarginine moiety modifying only to a minor extent the cardiotoxic quality of the genin. The effect on the heart is very similar to that of the digitaloid drugs, and the toxicity is of the same order of magnitude. Bufotoxin and digitoxin are about equally toxic, as judged by the different methods of assay, but there is a marked difference in that neither bufotoxin nor bufotalin has the persistency of action characteristic of digitoxin and the other cardiac glycosides. The persistency of action, which possibly is associated with the presence of the sugar groups, is an

⁹⁸ Wieland and Voëke, *Ann.*, **481**, 215 (1930).

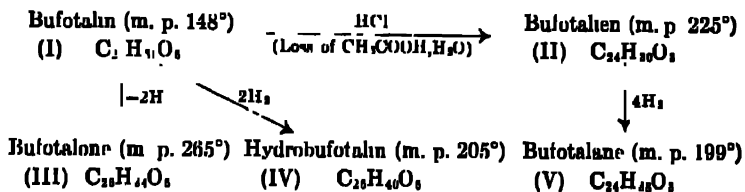
important quality in heart therapy, and it is perhaps because the active principles of the toad poisons lack this property that, from such clinical studies as have been conducted, they appear to possess little therapeutic value as compared with the digitaloid drugs.⁶⁵

Isolation of the Genins. Although the difficulty of obtaining large amounts of the toad poisons has necessarily limited the study of the occurrence of the active principles, some idea of the quantities of materials present in the skin secretions can be gained from the following estimates, which are based largely upon the investigations of Wieland. A single dried toad skin weighing about 15 g. yields about 7-10 mg. of the pure genin, or a correspondingly larger amount of the conjugated compound. From the glands of a single live toad there can be obtained by expression about 13 mg. of (dried) secretion, of which about 4.5 mg. can be recovered as crude genin and about 0.07 mg. can be obtained as pure bufotenine. A single Jamaican toad yields about 260 mg. of dried venom. When the dried skins are used as a source of material they are submitted to cold extraction with dilute alcohol for a period of several months. The organic solvent is evaporated in vacuum and replaced by water, which retains the basic constituents as salts and causes the genins to precipitate. A more satisfactory method of obtaining supplies of poison was developed by Abel and Macht and later used in the extensive investigations of Wieland at Freiburg and Munich. The toad is caught and the secretion contained in a pair of large glands located behind the eyes is expressed with flat forceps. The stream of milky fluid is caught in a bowl inverted over the toad, and the only protection required by the operator is a pair of eyeglasses. The animal is set free at the place of capture and suffers no injury. In a ten-day period it was possible to collect the secretion from 27,000 common toads found in the environs of Freiburg. There was no abundance of dead toads following the collection, and the number captured was very nearly the same in two succeeding years.

The Structure of Bufotalin. In his first work on bufotalin in 1913, Wieland⁶⁷ was impressed by the striking similarity of this substance to certain other natural products. The positive Liebermann-Burchard test was reminiscent of the sterols, while the peculiarly specific effect of the substance on the heart suggested a close relationship to the cardiac aglycones. Like these latter substances, bufotalin appears to contain a characteristic unsaturated lactone ring which is intimately associated with the physiological activity, for the toxic character is largely lost on hydrogenation. That a center of unsaturation is located in the lactone ring is indicated by the fact that the acid liberated on hydrolysis is so sensitive and alterable that it has thus far eluded isolation.

⁶⁷ Wieland and Well, *Ber.*, 46, 3315 (1913).

Further similarities can be seen in transformations which reveal the character of the other functional groups. The conversion of bufotalin (I) into a ketone (III) on oxidation, establishes the presence of a secondary hydroxyl group, which is also indicated by the formation from I of an



acetyl derivative. The absorption of two moles of hydrogen to produce the neutral substance IV proves the presence of two double bonds, while the formation of the yellow bufotalien (II) with the loss of acetic acid shows that bufotalin contains an acetoxyl group. Two additional double bonds are introduced in the production of bufotalien (II), as indicated by the composition of the hydro derivative (V), and the fact that bufotalien is colored suggests a condition of conjugation of the unsaturated linkages. That the original secondary hydroxyl group is still intact in V is shown by the oxidation of the substance to a ketone, bufotalanone.

Clearly bufotalin is the acetyl derivative of a doubly unsaturated trihydroxy lactone, and by 1920 careful analyses had established that the parent substance is a C_{24} -compound having four reduced rings.⁸⁸ This strongly indicated a close relationship to the bile acids, which also are C_{24} -compounds, and, after the method of mass extraction of poison from living toads had made available sufficient material for investigation, an attempt was made to bridge the gap between the two series.⁸⁹ If there is such a relationship a cholanic acid should result on the removal of the hydroxyl groups, hydrogenation, and cleavage of the lactone ring. The chief difficulty encountered was in opening the lactone ring in a satisfactory manner. This could not be done prior to hydrogenation because of the alterable character of the hydrolysis product, and in the saturated compound bufotalane (V) the lactone ring resisted attempts to achieve a reductive fission. Examples have been given elsewhere in this chapter of the reductive cleavage of lactone rings having a double bond in the proximity of the point of attachment of the oxide linkage (pages 279, 296), and a similar reaction served to overcome the difficulty in the present instance. It was found that on hydrogenating acetyl bufotalien in the presence of a special catalyst, a part of the material was converted into a saturated lactone, acetyl bufotalane, while a part yielded a nicely

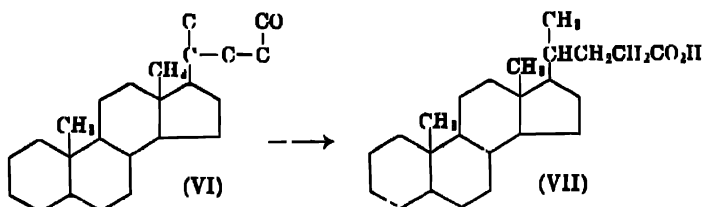
⁸⁸ Wieland and Weyland, *Sitzb. Bayer. Akad. Wiss.*, 329 (1920)

⁸⁹ Wieland, Heese and H. Meyer, *Ann.*, 493, 272 (1932)

crystalline acid having the composition of an acetoxycholanic acid. Hydrolysis gave a substance isomeric with the hydroxycholanic acids but not identical with any of the known substances of this group. In order to reduce the number of possibilities for isomerism the hydroxyl group was eliminated by pyrolytic dehydration to an unsaturated compound, followed by hydrogenation. The product was very similar to the four known cholanic acids, namely cholanic acid, *allo*-, urso-, and bufocholanic acid (page 127), but it was not identical with any of these substances. Wieland was inclined to believe that the difference is a stereochemical one and that the structures are the same. There is ample opportunity for steric rearrangements in the saturation of the four ethylenic linkages of bufotalin. Wieland named his degradation product isobufocholanic acid, and in 1935 he obtained, with Hesse,¹ further evidence indicating a bile acid ring system for bufotalin. On dehydrogenation with selenium, the substance (4 g.) yielded a small amount of a hydrocarbon identified as somewhat impure chrysene. Taken in conjunction with the great amount of accumulated information regarding the dehydrogenation of cholesterol, cholic acid, and oestrone, this evidence strongly supports an actiocholane ring system for the toad poison genin.

Support of this view has been presented by Miss Crowfoot in her work with bufagin.² An X-ray crystallographic study of bufagin gave the value 446 ± 9 for the molecular weight of the compound containing one molecule of alcohol of crystallization, which is in good agreement with the molecular weight, 446, calculated on the basis of the formula $C_{24}H_{32}O_7$. The X-ray measurements point clearly to an outline skeleton similar to that of the cardiac aglycones. Although bufagin and bufotalin have not yet been correlated, and although a definite proof of the ring system is still lacking, it is reasonable to suppose that the carbon skeleton for these toad poison principles conforms to the usual sterol pattern.

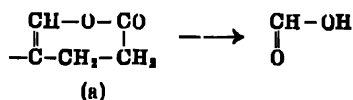
Accepting the structure VII for isobufocholanic acid, bufotalin may be assigned the outline formula VI. According to observations of Wieland



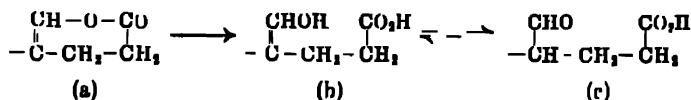
¹ Wieland and Hesse, *Ann.*, **517**, 22 (1935).

² Crowfoot, *Chemistry and Industry*, **54**, 569 (1935).

and Hesse,¹ the side chain very probably has the structure of a γ,δ -unsaturated δ -lactone (a), one important piece of evidence being the isolation of formic acid as a product of the ozonization of bufotalin:

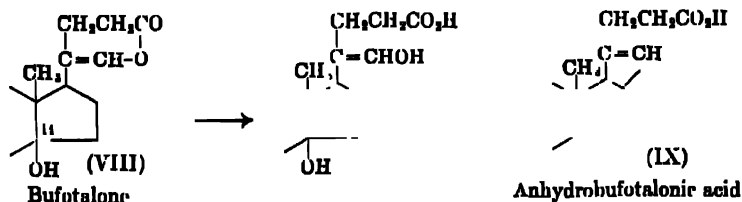


It is consistent with this formulation that the Legal nitroprusside test is negative, and the cleavage of the lactone ring under special conditions of hydrogenation also demands the location of a double bond in the side chain. The structure (a) represents the lactone of an aldo-enol acid, and the hydrolysis should proceed, at least in the first stages, as follows:



Because of the extreme sensitivity of the hydrolysis product to alkali, it was not easy to demonstrate the presence of the latent or free aldehydic group, (b) or (c), but this eventually was accomplished by conducting the hydrolysis in the presence of ammoniacal silver hydroxide solution or the Schiff reagent, when strong reducing properties were revealed.

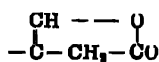
Another enlightening observation was made in the case of bufotalone (VIII), which forms a somewhat more stable acid on hydrolysis. On treatment with very dilute alcoholic alkali at 0° for 5 hours, there was obtained after acidification an anhydro compound (IX) having the character of an acid, but totally lacking in reducing properties. The conditions of the reaction are so mild that the original acetyl group is retained. Since the loss of water occurs without relactonization, it must result from the interaction of the aldehydic group with a conveniently located hydroxyl group of the ring system. The reaction closely resembles the isomerization of strophanthidin and of scillaridin A,



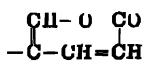
and by the usual reasoning it indicates that one of the hydroxyl groups of bufotalin probably is located at C₁₄.

The elucidation of the character of the side chain establishes a close structural relationship between the toad poison genins and the cardiac

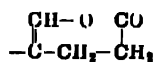
aglycones. According to the evidence available, strophanthidin, scillaridin A, and bufotalin contain similar groupings. In each case the action



Strophanthidin



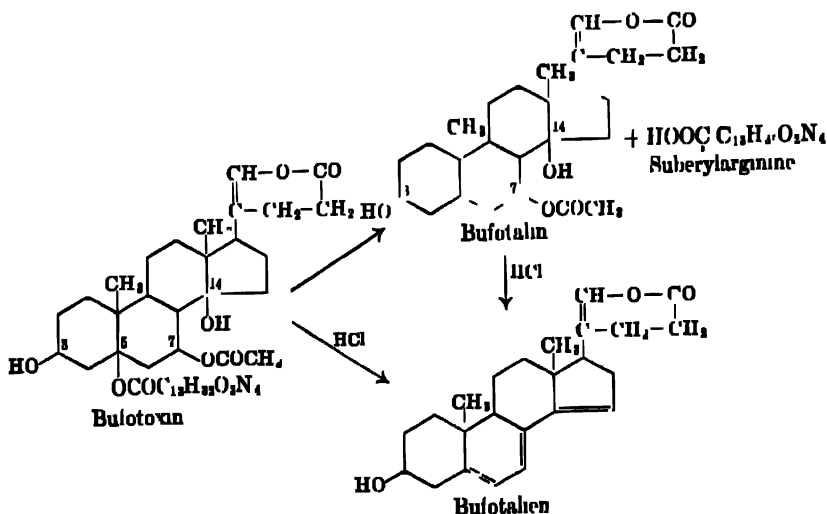
Scillaridin A



Bufotalin

on the heart appears to depend primarily upon the presence of the unsaturated lactone ring. Specifically, bufotalin is most closely related to scillaridin A, for both are derived from C_{14} -structures. If the present formulations of the side chains are correct, it may be possible to correlate bufotalin with the bile acids by way of scillaridin A. The saturated ketone bufotalanone of Wieland, Hesse and Meyer⁸⁹ on elimination of the carbonyl oxygen atom should give a substance having the structure, and possibly the configuration, of Stoll's saturated lactone (page 296).

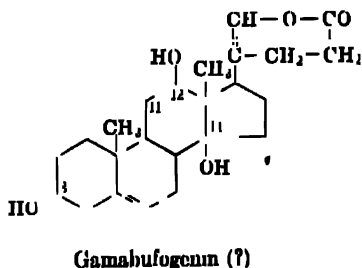
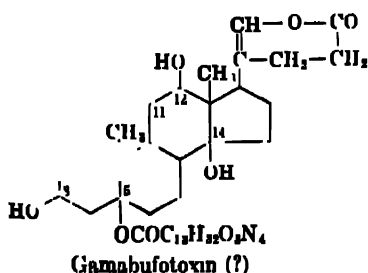
For bufotalin Wieland and Hesse¹ have suggested with reservation the structural formula reproduced in the chart below, but they regard this as but one of many possibilities. Although the information regarding the location of the various characteristic groups is assuredly meager, there are considerations which lend definite weight to this provisional formulation of the genin, and to the corresponding structure assigned to bufotoxin. The placing of the secondary hydroxyl group at C_3 is purely



arbitrary, but in analogy with the other natural products this is an inviting hypothesis. The other three characteristic groups of bufotoxin appear to be interrelated. When suberylarginine is cleaved from the molecule under very mild conditions, as in the animal organism, a double

bond is introduced into the ring system. This easy elimination of the acid residue is indicative of a tertiary linkage, as at C₅. Bufotalin, in turn, easily loses the elements of acetic acid and water, which is interpreted as showing that either the acetoxyl group or the tertiary hydroxyl group is in a position to be activated by the nuclear double bond. Since both groups are eliminated together in the formation of bufotalien they must bear the 1:3 relationship; when one is lost the new double bond labilizes the remaining group. Evidence cited above indicates that the unacetylated hydroxyl group interacts with the side chain and probably is located at C₁₄, and the acetoxyl group may be placed at the alternate C₇-position. The fact that the group which is present in acyl combination, and which therefore is probably secondary, is also easily eliminated (like a tertiary group), finds adequate explanation in the tendency to form a conjugated system. The formulation of bufotalien is consistent with the fact that it is colored. To recapitulate, three of the attached groups of bufotoxin appear to bear the 1:3:5 relationship, and the recognition that one of these groups is situated at C₁₄ locates the other two.

Gamabufogenin (C₂₄H₃₁O₇), m. p. 255°. This genin, isolated from the skins of Japanese toads by Wieland and Vocke,⁹⁶ has the composition of the parent substance of which bufotalin is the acetyl derivative, but the two series are different. While the poisons from *B. vulgaris* give unusually intense color displays in the Liebermann-Burchard reaction, the gamma compounds give only feeble tests. Wieland suggested that this may be because a less extensive unsaturated system is produced in the course of the reaction with acetic anhydride and sulfuric acid, and a relative stability is indicated clearly by the fact, already noted on page 305, that gamabufogenin forms only a monoanhydro compound under the influence of hot, concentrated hydrochloric acid. This is in marked contrast to the conversion of bufotalin by cold acid into the dianhydro derivative bufotalien, and it provides an important clue to the structural difference. The hydroxyl groups of gamabufogenin evidently are not distributed in such a way that the elimination of one group causes a second one to become labile. Since the genin forms a diacetyl derivative



(m.p. 252°) two of the groups are secondary, and the group capable of being eliminated is probably tertiary. The provisional formulas shown for gamabufogenin and its conjugated compound, gamabufotoxin, may be suggested as meeting all of the known requirements.

According to this formulation the chief difference between bufotalin and gamabufogenin is that one of the secondary hydroxyl groups of the latter substance is located in ring C rather than in ring B. This provides an explanation of its failure to become labile following the production of an anhydro linkage on the elimination of the tertiary hydroxyl group at C₁₄. In the formulas the group in question has been placed at one of the bile acid positions, C₁₂, but an equally possible location is at C₁₁.

The venom of the Japanese toad *B. vulgaris formosus* was also investigated by Kotake³ who obtained from the dried skins of some 5,000 toads 30-40 g. of a genin which he at first regarded as a new substance ("gamabufotalin, C₂₇H₄₄O₆"), but which he later⁴ recognized as identical with gamabufogenin.

The Active Principles of Ch'an Su. There have been a number of conflicting reports regarding the cardiac principles of Chinese toads and of the drug Ch'an Su and the problem is at present too unsettled to warrant any but a brief summary of some of the more significant observations.

Jensen and Chen⁵ isolated from Ch'an Su a substance melting at 223° (acetate, m.p. 196°) to which they assigned the formula C₂₅H₃₂O₆ or C₂₀H₃₄O₆ and the name **cinobufagin**. Cinobufagin apparently is identical with Kotake's "bufagin"⁶ (m.p. 221°; acetate, m.p. 197°) from the same source. X-ray measurements of Miss Crowfoot² indicate a molecular weight of 447 ± 10, which agrees best with the C₂₀-formula (442). The substance appears to be the acetyl derivative of a C₂₄-compound, acetic acid being liberated on hydrolysis. The presence of a lactone ring, a secondary and a tertiary hydroxyl group, and two double bonds is indicated (Kotake, Jensen⁷). In the secretion the substance is conjugated with suberylarginine (Jensen and Chen). Pharmacological studies of Chen and Chen⁸ indicate that the drug Ch'an Su probably is prepared from the Chinese toad *Bufo bufo gargarizans*. Recently Tschesche and Offe⁹ confirmed the composition and the melting point

² Kotake, *Ann*, **465**, 11 (1929). See also K. K. Chen, Jensen and A. L. Chen, *J. Pharmacol*, **49**, 26 (1933).

³ Kotake, *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **24**, 39 (1934).

⁴ Jensen and K. K. Chen, *J. Biol. Chem.*, **87**, 741 (1930).

⁵ Kotake, *Ann*, **465**, 1 (1929).

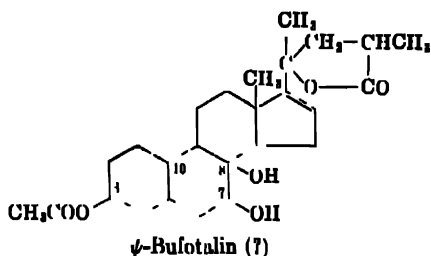
⁶ Jensen, *Strimer*, **75**, 53 (1932).

⁷ K. K. Chen and A. L. Chen, *J. Pharmacol*, **49**, 543 (1933).

⁸ Tschesche and Offe, *Ber*, **68**, 1998 (1935).

reported for cinobufagin by Jensen and Chen. These investigators subjected the genin to dehydrogenation with selenium and obtained the Diels hydrocarbon, thus identifying the ring system with that of the sterols.¹⁰ They report that 2.5 kg. of Ch'an Su yielded 15 g. of cinobufagin.

Kondo and Ikawa¹¹ have investigated extensively an apparently different substance from Ch'an Su and from Chinese toads. The substance was at first thought to be identical with Wieland's bufotalin, but differences became apparent on further investigation and the compound was called **pseudobufotalin**. The substance is said to sinter at 107° and to melt at 145-146°; it is assigned the formula $C_{26}H_{36}O_6$ and regarded as the acetyl derivative of a C_{24} -compound. In the most recent report, Ikawa has suggested for ψ -bufotalin a formula which he regards as estab-



lished in all points except the location of the acetoxyl group and the secondary hydroxyl group. The principal lines of evidence cited, besides a general characterization of the functional groups, are the isolation of the Diels hydrocarbon as a product of dehydrogenation and the systematic degradation of the side chain of desacetyltetrahydro- ψ -bufotalin by successive Grignard reactions and oxidations. This was extended to include the opening of the five-membered ring, and the ability of an acidic degradation product to form a lactone ring with the tertiary hydroxyl group provided evidence that this is located at C_8 .

The experimental details of this important work have been published only in the Japanese language and the author has been unable to evaluate the evidence from the summaries in German. If the claims are indeed fully substantiated, ψ -bufotalin differs from all other cardiac principles in having a saturated, rather than an unsaturated, lactone ring. Further remarkable features of the suggested formula are that the side chain skeleton does not conform to that of any known sterol and that the usual angular methyl group is missing at C_{10} . The latter point appears highly uncertain, since it is based purely on analytical data.

¹⁰ The observation was confirmed by Jensen, *J. Am. Chem. Soc.*, **57**, 2733 (1935).

¹¹ Kondo and Ikawa, *J. Pharm. Soc. Japan*, **53**, 2, 62 (1933); **54**, 22 (1934); Ikawa, *ibid.*, **55**, 40, 144 (1935).

According to Kobayashi¹² the cardiotonic activity of ψ -bufotalin in cats is about one twenty-fifth that of ouabain. Chen and Chen¹³ found cinobufagin to be about one-half as active as ouabain in the cat test.

Bufagin ($C_{24}H_{32}O_5$), m.p. 213°. Bufagin was first isolated from the tropical American toad *B. aqua* (*B. marinus*) by Abel and Macht in 1911, but no chemical characterization of the substance was undertaken. Later Jensen and Chen¹⁴ isolated an identical substance from the same source and on the basis of closely agreeing analyses of the genin and its monoacetyl derivative (m.p. 204°) they suggested the formula $C_{23}H_{30}O_5$. Jensen⁷ later made a preliminary report of some of the reactions of bufagin, and stated that on treatment with either acids or bases a molecule of formic acid was liberated. Noting the close similarity in chemical and pharmacological properties to the cardiac aglycones, all of which at the time were regarded as C_{23} -compounds, Jensen suggested that bufagin is the formyl derivative of a C_{23} -compound. The formula $C_{24}H_{32}O_5$ is in as good agreement with the analytical data as the earlier formula, and it has been substantiated by the X-ray work of Miss Crowfoot,² but the relationship to the plant heart poisons is not that suspected when the formula was proposed.

In 1934 Jensen and Evans¹⁴ described further some of the transformations of bufagin. The substance is a lactone, and the formation of a monoacetyl derivative indicates the presence of a secondary hydroxyl group. On catalytic hydrogenation bufagin is converted chiefly into a tetrahydro derivative (m.p. 211°) showing that two double bonds are present and there is also produced a small amount of an acidic compound which possibly results from the reductive cleavage of an unsaturated lactone ring. The formation of a dianhydro compound under the influence of boiling, dilute alcoholic sulfuric acid suggested the presence of two tertiary hydroxyl groups.

Reinvestigating the action of boiling alcoholic alkali on bufagin, Jensen and Evans obtained, after acidification, an amorphous acid (bufaginic acid) to which they assigned the formula $C_{21}H_{34}O_6$, indicating the loss of one carbon atom. The other product of hydrolysis was identified as formic acid, but it was pointed out that this may arise from formaldehyde through the Cannizzaro reaction. While mild treatment with acids gives, if in poor yield, dianhydrobufagin, it was found that on treating bufagin with 50% sulfuric acid at 70°, formaldehyde could be detected as a cleavage product. The other product was not identified. On the basis of this observation, and in view of the evidence that other toad poisons are C_{24} -compounds, the earlier idea of a formylated C_{23} -

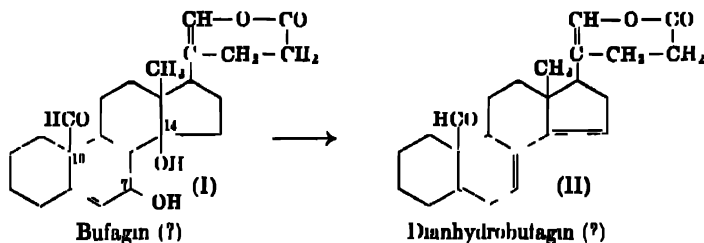
¹² Kobayashi, *Proc. Imp. Acad. (Tokyo)* 11, 298 (1935).

¹³ Jensen and K. K. Chen, *J. Biol. Chem.*, 87, 755 (1930).

¹⁴ Jensen and E. A. Evans, Jr., *ibid.*, 104, 307 (1934).

derivative was abandoned. In analogy with the acetolysis of ouabain (page 291), in which formaldehyde is liberated, Jensen and Evans adopted the explanation suggested in the other case by Jacobs and Bigelow, namely that the formaldehyde comes from a doubly bound methylene group. This explanation appears no more appropriate here than in the case of ouabain. The cleavage reaction probably involves a rearrangement and should be regarded as evidence of only secondary importance.

The X-ray work affords such a convincing indication of the outline structure of bufagin that some speculation regarding the possible nature and location of the functional groups appears justified. It is suggested that formulas I and II for the genin and its dianhydro derivative account



for all of the observations reported. The ready elimination of two hydroxyl groups, one of which is secondary, is attributed, as in the case of gitoxigenin (page 280), to the special 1:3 relationship between them. The presence of an angular aldehydic group is inferred chiefly from the accurately established empirical formula (and the apparent absence of additional hydroxyl groups capable either of acetylation or of elimination). The liberation of formaldehyde under certain conditions may be the result of an aromatization of ring B with the expulsion of the aldehydic group.

Other Toad Poisons. Investigations of the skin secretions of various other species of toads indicate the existence of still other poisonous principles. The substances listed in the table, page 316, apparently have been isolated in a pure condition, but only preliminary characterizations have been reported. Queriericobufagin and veridobufagin are similar in properties and possibly identical, but a direct comparison has not been made.

Although the problem of the structure of the toad poisons has not been solved completely in any one case, the work has progressed sufficiently far to show that there are a number of different members of the group and that they are more or less closely related to one another and to the plant heart poisons. The most fully characterized toad poison appears

Source of toad	Genin	M.p.	Probable formula	Derivatives, m.p.
Argentina	Arenobufagin ^{15,16}	220°	C ₂₅ H ₃₄ O ₆	Monoacetate, 163°
South Africa	Regularobufagin ^{10,17}	235-230°	C ₂₅ H ₃₄ O ₆	Monoanhydro compound, 255°
North America	Vallicepobufagin ¹⁸	212-213°	C ₂₀ H ₃₈ O ₅	
{ North America	Quecriceobufagin ¹⁸	258-259°	C ₂₃ H ₃₁ O ₅	
{ Europe	Veridobufagin ¹⁹	255-255.5°	C ₂₃ H ₃₄ O ₅	Diacetate, 254°

to have the same skeletal structure as the bile acids, and this is true also of scillaridin A. The majority of the cardiac aglycones have one less carbon atom in the side chain. The most distinguishing characteristic of the cardiac principles, in comparison with the bile acids, is that the sterol side chain is hydroxylated, as well as being oxidized to an acid group, and that the two functional groups are combined in the form of a lactone ring. The lactone ring, which almost invariably is unsaturated, is largely responsible for the physiological action. Another interesting difference is that the poisonous principles usually contain tertiary hydroxyl groups in the nucleus, in addition to the secondary groups characteristic of sterols and bile acids. From such evidence as is available it seems likely that the cardiotonic substances are products of the biological oxidation of sterols. Clearly the origin and function of the poisons and the problem of correlating the structures and physiological actions of these interesting substances offer a most inviting field for further investigation.

¹⁵ K. K. Chen, Jensen and A. I. Chen, *J. Pharmacol.*, **49**, 1 (1933).

¹⁶ Jensen, *J. Am. Chem. Soc.*, **57**, 1765 (1935).

¹⁷ K. K. Chen and A. I. Chen, *J. Pharmacol.*, **49**, 503 (1933).

¹⁸ *Ibid.*, **49**, 526 (1933).

¹⁹ K. K. Chen, Jensen and A. I. Chen, *ibid.*, **49**, 11 (1933).

Chapter VII

Saponins

Substances of plant origin having, like soaps, the property of forming colloidal aqueous solutions which foam on shaking are known as saponins. The class is a broad one, for substances of several different chemical types and having various distinct actions on the animal organism conform to the above definition of a saponin. The plant heart poisons are saponins and the toad poisons are at least saponin-like compounds, but these substances have distinctive physiological properties which set them apart from those with which the term saponin is most generally associated. In the following description of the properties of these other saponins, the observations apply only to the restricted group exclusive of the cardiac poisons.

The saponins are glycosidic substances which, in addition to their foam-forming action, are recognizable by their ability to bring about the hemolysis of red blood corpuscles even at high dilutions. Many have a bitter taste, cause sneezing, and irritate the eyes. Among the more abundantly occurring saponins are those found as mixtures in soapwort, soaproot, soapbark, snake root, smilax, and plants of the gourd family. Considerably rarer are the saponins found in digitalis plants along with the cardiac glycosides. Crude saponin mixtures are readily obtainable from the more abundant sources, but the isolation of the pure glycosides is a problem of unusual difficulty. Saponin mixtures, or parts of the plants themselves, have found various uses by virtue of the ability of the glycosides to produce a soapy lather in aqueous solution. The plants have been employed since earliest times as soaps, fish poisons, and remedies. Unlike ordinary soaps, the saponins are not salts and they are not precipitated in hard water. Since the solutions do not give an alkaline reaction, saponins at one time were preferred for washing delicate fabrics and for use in the presence of sensitive dyes. Saponin-containing plants were employed by primitive peoples to poison fish without rendering them inedible. The fish are either dazed or killed when the saponin is introduced by macerating the plant in the water. Aqueous extracts of sarsaparilla root from various species of *Smilax* have been used medicinally for several centuries. Saponins have found some use as foam producers in fire extinguishers and, in admixture with ordinary soaps, in the manufacture of shaving preparations. Small quantities of saponins taken

by mouth do not appear to be harmful, probably because they are not absorbed through the intestinal wall.¹

While very few saponins are known in a pure condition, extensive characterizations have been made of a number of their hydrolysis products, the sapogenins. These are all hydroxylated polynuclear compounds and some of them contain acidic groups. Recent dehydrogenation studies have shown that the sapogenins can be divided into two classes, those which, like certain polynuclear triterpenoid compounds, yield mixtures of aromatic hydrocarbons rich in naphthalene derivatives, and those which are converted into the Diels hydrocarbon and which therefore have sterol-like structures. The sapogenins of the second group clearly are related in structure to the cardiac aglycones and some of them, in the form of the saponins, occur with the heart poisons in digitalis plants. Saponins of the digitalis group are the only ones which have been characterized as chemical individuals and they will be described below in a separate section preceding a discussion of the sterol-like sapogenins.

Robert's original classification of the saponins as acidic and neutral according to the behavior of the substances themselves has been largely abandoned because the knowledge of the pure substances is too meager and because the differences are not sharp.² A classification of saponins based on the nature, acidic or neutral, of the aglycones obtained on hydrolysis, was for a time employed by Tschesche,³ but this appears less reliable than that based on the results of the dehydrogenations. It is true that most sapogenins of the type related to triterpenes contain acidic groups, and that the few sapogenins known to have sterol-like structures are neutral substances. There is, however, at least one doubtful case in the first group, aescigenin being a neutral sapogenin, and there probably are other exceptions which still await investigation.

TRITERPENOID SAPOGENINS

The recognition that certain of the sapogenins belong to a separate chemical group was due to Ruzicka.⁴ On dehydrogenation with selenium at a high temperature the sapogenins of this group yield similar mixtures of hydrocarbons containing, in every case investigated, the characteristic degradation product sapotalene. This substance has been identified as 1,2,7-trimethylnaphthalene, and its regular formation from a large number of natural products suggests a common feature in their molecular structures

¹ Tschesche, *Z. angew. Chem.*, **48**, 560 (1935)

² L. Koffer, "Die Saponine," J. Springer, Vienna, 1927

³ Tschesche, *Ber.*, **68**, 1090 (1935)

⁴ Ruzicka and co-workers, *Helv. Chim. Acta*, **15**, 431, 1476 (1932), **17**, 442 (1934)

The sapogenins are found in combination with various sugars. Hederin ($C_{41}H_{84}O_{11}$), for example, yields hederagenin ($C_{30}H_{48}O_4$), rhamnose ($C_6H_{12}O_5$), and arabinose ($C_5H_{10}O_5$). Acid radicals are sometimes held to the sapogenin moiety in ester linkage, as in the case of aescin ($C_{55}H_{88}O_{27}$) from horse chestnut, which is hydrolyzed by acids to glucose, glucuronic acid, and aescigenin ($C_{35}H_{58}O_7$), but the latter aglycone is not the fundamental unit, for with alcoholic alkali it is further cleaved to tiglic acid ($C_5H_8O_2$) and a C_{30} -compound. Some of the other sapogenins which yield sapotalene on dehydrogenation are: camelliasapogenin, caryocarsapogenin, cyclamiretin, glycyrrhetic acid, gypsogenin, mimusopassapogenin, panaxsapogenin, quillaiasapogenin (Ruzicka and co-workers⁵), and echinocystic acid.⁶ It appears probable that in each case the basic unit, after the hydrolysis of all sugar and acid groups, is a C_{30} -compound.

Closely related to these sapogenins are certain acids of plant origin which are not known to occur as glycosides but are found in the uncombined state. Examples are: ursolic acid ($C_{30}H_{48}O_3$), elemolic acid ($C_{30}H_{48}O_3$), boswellinic acid ($C_{32}H_{52}O_4$), and the isomers suma- and siarresinolic acid ($C_{30}H_{48}O_4$). Oleanolic acid ($C_{30}H_{50}O_3$), one of the most widely distributed members of the group, has in some instances been found combined with glucuronic acid and so forms a bridge between the sapogenins proper and these other substances. A close structural relationship is indicated by the fact that all of these acids yield sapotalene on dehydrogenation with selenium, and the acids may be classed as sapogenins.

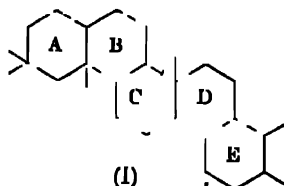
Those sapotalene-yielding sapogenins which have been most accurately characterized are all C_{30} -compounds, which suggests a relationship to the triterpene alcohols, such as amyrine ($C_{30}H_{50}O$) and betulin ($C_{30}H_{50}O_2$). Such a relationship has been well established by the observation that these alcohols yield sapotalene and other dehydrogenation products identical with those obtained from the above mentioned acids, and this has led to the recognition of the sapogenins of this type as triterpene acids. The most fully characterized products of the dehydrogenation of the various triterpene alcohols and acids are as follows:

- 1,2,3,4-Tetramethylbenzene
- 2,7-Dimethylnaphthalene
- 1,2,7-Trimethylnaphthalene (Sapotalene)
- 1,2,7-Trimethylnaphthol
- 1,2,5,6-Tetramethylnaphthalene
- A pentamethyldinaphthyl, $C_{25}H_{24}$
- A picene homologue, " $C_{25}H_{24}$ "

⁵ For references see Ruzicka, Brüngger, Egli, Ehmann, Furter and Uehli, *Helv. Chim. Acta*, 15, 431 (1932).

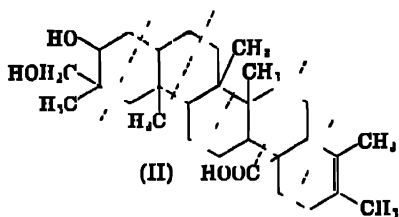
⁶ Bergsten and Noller, *J. Am. Chem. Soc.*, 56, 1403 (1934).

In the case of sumaresinolic acid all of these products of fission and dehydrogenation were isolated,⁷ while echinocystic acid yielded all but the dinaphthyl homologue.⁸ With this information as to the probable nature of the primary fragments resulting from the rupture of the C₃₀-structure, and making use of the isoprene rule, Ruzicka has postulated for the triterpenoids the carbon skeleton I. Sapotalene may come from the

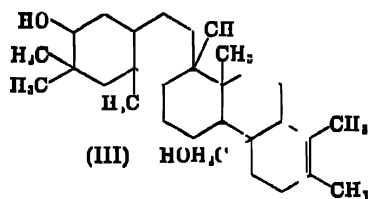


grouping AB, following cleavage in the manner indicated by the dotted line. A slightly different cleavage could yield 2,7-dimethylnaphthalene from rings A and B. 1,2,3,4-Tetramethylbenzene may arise from any of the rings B, C, D, and E. The dinaphthyl derivative may contain rings A and B singly linked to rings D and E, while dehydrogenation without ring rupture would account for the formation of a picene homologue.

On the basis of these and other observations, Ruzicka⁹ has suggested for some of the triterpenoids provisional formulas (II and III) embodying this skeleton. The dotted lines in his formula for hederagenin (II)



Hederagenin



Betulin

indicate the six isoprene units. In the case of oleanolic acid there is good evidence, particularly from the work of Kitasato¹⁰ and of Wedekind,¹¹ that the substance is a pentacyclic hydroxy- γ,δ -unsaturated acid with a tertiary carboxyl group. Formulas IV and V, both of which comply with the isoprene rule, are under consideration at the time of this writing.

While the knowledge of the structures of even the more prominent members of the group is still incomplete in many respects, the work has

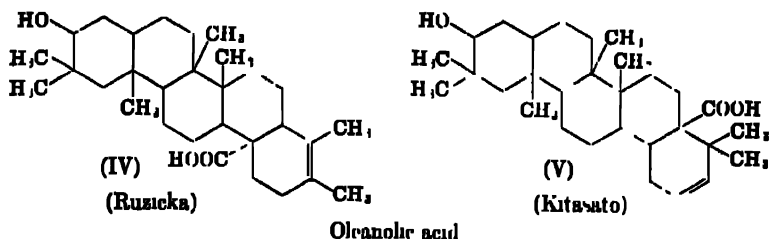
⁷ Ruzicka and Hübl, *Helv. Chim. Acta*, **15**, 448 (1932), **17**, 453 (1934)

⁸ Noller, *J. Am. Chem. Soc.*, **56**, 1582 (1934)

⁹ Ruzicka, *Ann. Rev. Biochem.*, **3**, 459 (1934)

¹⁰ Kitasato, *Acta Phytotchem. (Japan)*, **6**, 179, 223, 305 (1932), **7**, **1**, 170 (1933), **8**, **1**, 207, 255, 315 (1934)

¹¹ Wedekind and Schickel, *Z. physiol. Chem.*, **195**, 132 (1931), **215**, 199 (1933), Aumüller, Schickel and Wedekind, *Ann.*, **517**, 211 (1936)



progressed far enough to show clearly that the sapogenins in question are triterpene acids probably containing a reduced piceene ring system, and that they are higher members of the structural series of which abietic acid is a typical representative. There is no indication of either a structural or a biogenetic relationship to the sterols and the other actiocholane derivatives, and consequently this interesting group of substances will be left with no more than this brief reference to the type of structure which they embody.

SAPONINS OF THE DIGITALIS GROUP

The only members of the group which have been fully characterized as chemical individuals are digitonin ($C_{76}H_{122}O_{29}$) and gintonin ($C_{70}H_{122}O_{23}$). These substances occur in digitalis seeds along with at least one other saponin (tigonin) and in conjunction with the cardiac glycosides of digitalis. The isolation of a pure saponin of this or other type is complicated by adverse physical properties, by the sensitivity of the glycoside, by the absence of a sharp and characteristic melting point, and by the presence of other, closely related substances. These circumstances have led to much confusion and error in the literature, and relatively little is yet known concerning the individual components of most of the saponin mixtures. Digitonin, the best characterized of all of the saponins, is a rare and expensive substance. The glycoside on hydrolysis yields four molecules of hexoses, one molecule of xylose, and the aglycone digitogenin. The difficulty of obtaining the pure material can be appreciated from the fact that chemical investigations were carried out for a period of many years with preparations which were later found to contain no more than about 75% of pure digitonin. In 1913 Windaus and Schnackerburger¹² discovered the presence in well purified "digitonin" of 10-20% of a second glycoside which they called gintonin, and which was found to yield a different sapogenin. Both glycosides can be crystallized from alcohol-water mixtures, and in each case the solubility passes through a minimum on going from absolute alcohol to 50% alcohol. The concentration of minimum solubility differs in the two cases, however,

¹² Windaus and Schnackerburger, *Ber.*, 46, 2628 (1913)

and gitonin is less soluble in 95% alcohol than digitonin, while the relationship is reversed in 85% alcohol. This situation affords a means of separating the two glycosides. Windaus¹³ later discovered another method based upon the rate of precipitation with ether from an aqueous solution. When a cold, 5% solution of crude digitonin in water is treated with ether, the digitonin precipitates rather rapidly while the precipitation of gitonin is very slow. The precipitation of digitonin becomes still more rapid when, after several repetitions, the product approaches complete purity. Windaus isolated degradation products other than those coming from digitonin or gitonin, and concluded that still other saponins are present in the original mixture from digitalis seeds. Jacobs and Fleck¹⁴ isolated from *D. purpurea* leaves the aglycone of another saponin, tigonin.

The most striking property of digitonin and the other saponins is the hemolytic action on red blood corpuscles. Even at high dilutions the addition of digitonin to defibrinated blood in physiological salt solution causes the rapid destruction of the erythrocyte structure with the liberation of hemoglobin. A possible explanation of the hemolytic effect is that the saponin combines with cholesterol or lecithin of the cell membrane and renders it pervious to the passage of hemoglobin. That such a combination is possible, at least in the case of cholesterol, is known with certainty from the work of Ransom,¹⁵ who in 1901 made the important discovery that treatment of a saponin solution with cholesterol destroys its hemolytic activity. Other hemolytic poisons can be detoxified in a similar manner. The studies of Ransom and others indicated that cholesterol is capable of combining with saponins, and in 1901 Windaus¹⁶ established the nature of the interaction. Digitonin and cholesterol combine in the proportion 1:1 to form a remarkably stable and sparingly soluble molecular compound, cholesterol digitonide. This substance is devoid of hemolytic properties.

The remarkable ability of digitonin to form molecular compounds finds its most important chemical applications in the sterol series (see page 118), but many simpler substances also combine with the saponin. The ethyl alcohol compound has little stability, for digitonin crystallizes from aqueous alcohol with water of crystallization rather than alcohol. With amyl alcohol a 1:1 compound is formed which crystallizes with six molecules of water. The compound has been employed in purifying digitonin, the glycoside being recovered after eliminating the alcohol by distillation with steam. Compounds are also formed with terpene alcohols,

¹³ Windaus, *Z. physiol. Chem.*, **150**, 205 (1925).

¹⁴ Jacobs and Fleck, *J. Biol. Chem.*, **88**, 545 (1930).

¹⁵ Ransom, *Deut. med. Wochschr.*, **27**, 184 (1901).

¹⁶ Windaus, *Ber.*, **42**, 238 (1909).

phenols, and thiophenols.¹⁷ When ether is added to an aqueous solution of digitonin, the glycoside separates as an ether compound, and ketones also appear capable of combining with the saponin. An interesting use of the digitonides is in the resolution of racemic alcohols.¹⁸ In the case of α -terpineol and *ac*-tetrahydro- β -naphthol the combination of (levo) digitonin with the levo alcohol proved to be less soluble than that with the *d*-form and an almost complete resolution was achieved.

SAPOGENINS RELATED TO THE STEROLS

The sapogenins are far more easily isolated in a pure form than are the saponins. They are sparingly soluble in water and soluble in various organic solvents, they are less alterable than the glycosides and more easily characterized by ordinary methods of identification. Certain sapogenins have been isolated whose glycosides are either unknown or of doubtful purity, but the number of well characterized genins is still very small. The principal compounds of the series are listed in the accompanying table, together with the chief references to the literature describing their isolation and early characterization.

Not long after the first isolation of digitonin by Schmiedeberg in 1875, Kiliani commenced a series of investigations of the digitalis saponins and sapogenins which was to extend over a period of many years. Kiliani's method of attack was by oxidative degradation, for he hoped that by some method of oxidation, or combination of methods, it might be possible to resolve the complicated aglyconic molecules into known products. This hope was not realized, and although Kiliani contributed much valuable information regarding methods of obtaining a number of different oxidation products, particularly in the digitogenin series, he gave little attention to the matter of analysis, and the empirical formulas which he suggested for the acids were in many cases grossly incorrect. The whole problem of correlating the different oxidation products and interpreting the results in terms of the structure of the sapogenin remained obscure until the matter was reinvestigated by Windaus. Windaus' interest in the field originated in his work for the doctorate in 1899 with Kiliani at Freiburg on the subject of the cardiac glycosides and saponins of digitalis.

From the results of careful analyses of digitogenin and gitogenin, and of a number of their degradation products and derivatives, Windaus assigned to the sapogenins the formulas $C_{26}H_{42}O_7$ and $C_{26}H_{42}O_4$, respectively. It should be noted that it is hardly possible by ordinary analysis to distinguish between the C_{26} -formulas and the corresponding C_{27} -

¹⁷ Windaus and Weinhold, *Z. physiol. Chem.*, **126**, 298 (1923).

¹⁸ Windaus, Kluhnardt and Weinhold, *ibid.*, **126**, 308 (1923).

SAPONINS AND SAPOGENINS

Saponin		Sapogenins			Sugar
Source	Probable formula	M.p.	Probable formula	M.p.	
Digitonin	<i>D. purpurea</i>	235°	Digitogenin ^a	253°	2 Glucose, 2 Galactose, Xylose
Gitonin	<i>D. purpurea</i> , <i>D. germanicum</i>	272°	Gitogenin ^a	272°	3 Galactose, Pentose
Tigogenin	<i>D. lavata</i> , <i>Chlorogalum pomaridianum</i>	260°	Tigogenin ^a	204°	2 Glucose, 2 Galactose, Xylose
Amolonn	<i>Chlorogalum pomaridianum</i>		Tigogenin		3 Glucose Galactose 2 Rhamnose
Sarsapogenin (?) Parillin (?)	<i>Radix sarsaparillae</i> , <i>Smilax</i> <i>Similar</i> <i>cinna</i> <i>Chlorogalum pomaridianum</i>		Sarsapogenin ^a (Parigenin) Smilagenin Chlorogenin ^a	199° 194° 276°	3 Glucose (?)

^a Kiliaci, *Ber.* 23, 1555 (1930); 24, 339 (1931); 43, 3562 (1910); 49, 701 (1916); 51, 1613 (1918); Kiliaci and Mark, *ibid.* 54, 3592 (1901); Kiliaci and Schweisinger, *ibid.* 57, 1215 (1904); Windaus and Willerdine, *Z. physiol. Chem.*, 143, 33 (1925); Windaus and Shah, *ibid.*, 151, 86 (1926).

^a Windaus and Schneckenburger, *Ber.* 46, 2623 (1913); Windaus and Lavert, *Z. physiol. Chem.*, 147, 275 (1925). See also Ref. 1.

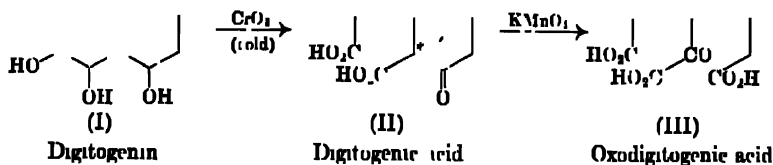
^a Jacobs and Fleck, *J. Biol. Chem.*, 86, 545 (1930); Windaus and Willerdine, (*loc. cit.*).

^a Fower and Salway, *J. Chem. Soc.*, 105, 201 (1914); Kaufmann and Fuchs, *Ber.* 56, 2527 (1923); van der Haar, *Rec. trav. chim.*, 48, 726 (1929); Jacobs and J. C. E. Simpeon, *J. Biol. Chem.*, 105, 501 (1934).

^a Liang and Noller, *J. Am. Chem. Soc.*, 57, 525 (1935).

formulas, for the differences amount to no more than 0.4-5% of carbon and 0.20-24% of hydrogen. Evidence to be presented below now favors the C_{27} -formulas given in the table, but the more important relationships established by Windaus apply equally well to either formulation.

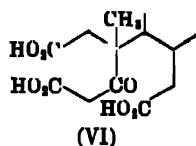
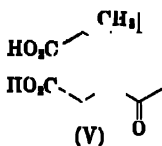
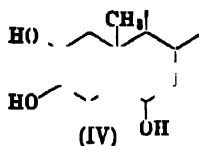
Of the five oxygen atoms of digitogenin, two are present in inert oxidic combination and the remaining three atoms occur as secondary hydroxyl groups, the sapogenin forming a triacetyl derivative. In 1925 Windaus and Willerding²⁴ were led to conclude that two of the groups in question occupy vicinal positions in one ring of the molecule and that the third is located in the α -position of an adjacent ring. The formation of Kiliani's digitogenic acid was represented as shown in the partial formulas I and II, the remainder of the molecule remaining unaltered.



The production of a dibasic keto acid (II) with the simultaneous destruction of two secondary hydroxyl groups proves that these groups occupy adjacent positions. The formula for digitogenic acid (II) accounts for the fact, noted by Kiliani and studied particularly by Windaus and Weil,²⁵ that the acid is easily converted by alkali into the more stable stereoisomeric, digitoic acid. As in the case of dehydrohydroxycholeic acid, enolization involving the asymmetric carbon atom adjacent to the carbonyl group doubtless is responsible for the inversion. On oxidation with permanganate both digitogenic acid and digitoic acid give a tribasic keto acid, oxodigitogenic ("oxydigitogenic") acid (III), without loss of carbon atoms. Oxodigitogenic acid has the properties of an α -keto acid, for it loses carbon monoxide when heated with concentrated sulfuric acid and the ester liberates the same gas when subjected to distillation. The properties of digitogenic acid, however, are not as easily reconciled with the formulation of the substance as a β -keto acid, for it is remarkably stable. With boiling alkali the acid is isomerized to digitoic acid without decarboxylation. Windaus and Willerding accepted the view that digitogenic acid belongs to the small, but not wholly unknown, class of stable β -keto acids largely because the alternate interpretation, expressed in formulas IV-VI, seemed even less plausible. If the ring had opened between β -positions in the terminal ring the second product of oxidation would be a β -keto acid (VI), but the substance not only lacks

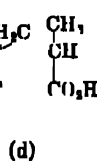
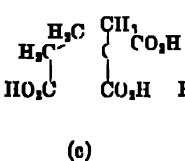
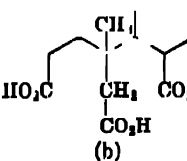
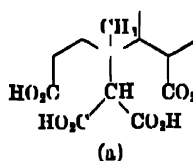
²⁴ Windaus and Willerding, *Z. physiol. Chem.*, **143**, 33 (1925)

²⁵ Windaus and Weil, *ibid.*, **121**, 62 (1922)



the properties ordinarily associated with such a structure but exhibits definite characteristics of an α -keto acid.

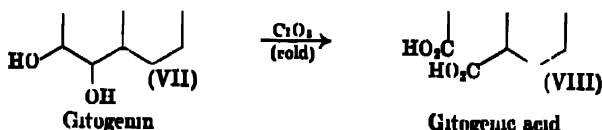
There are certain other indications in support of the formulation II for digitogenic acid. By the drastic oxidation of digitogenic acid with chromic anhydride, Windaus and Willerding obtained, among other products, a pentabasic "acid A" in which oxidation has occurred at various parts of the molecule. Anticipating evidence to be presented below, it may be said that the acid probably contains the fragment (a), which clearly can arise from II by a cleavage of the bond extending to



the right of the carbonyl group. Carbon dioxide is readily lost on heating "acid A" with acetic and sulfuric acids giving a substance with the grouping (b) [tetrabasic acid " $C_{25}H_{39}O_{10}$ "], and a product of the further oxidation is α -methylglutaric acid (d), which probably is formed through the α -carboxylic acid derivative (c). When α -methylglutaric acid was first isolated as an oxidation product it was taken for a fragment of the side chain, but present indications suggest that it arises from a part of the aglycone molecule corresponding to that involved in the formation of the same degradation product from desoxycholic acid (page 153). The alternate formulas for digitogenin and digitogenic acid, IV and V, would not allow for the formation either of a substance with a malonic acid grouping (a) or α -methylglutaric acid.

Gitogenin, a second sapogenin of digitalis plants, has but two secondary (acetylable) hydroxyl groups and the remaining two oxygen atoms appear to be in oxidic combination. On gentle oxidation gitogenin yields a dibasic, hydroxyl-free acid, indicating a vicinal arrangement of the groups attacked simultaneously in the process. The reaction was represented by Windaus and Linsert²⁰ (1925) as in formulas VII and VIII. On oxidation of gitogenic acid under more drastic conditions the part of the molecule containing the two acidic groups apparently remains intact,

²⁰ Windaus and Linsert, *Z. physiol. Chem.*, 147, 275 (1925)



while oxidation occurs in the neighborhood of the oxide rings. With nitric acid Windaus and Linsert²⁶ obtained a dibasic lactone acid of the formula $\text{C}_{22}\text{H}_{32}\text{O}_8$. Obviously a portion of the molecule is eliminated in the course of the reaction, and it is probable that the two carboxyl groups correspond with those of gitogenic acid. On the basis of this assumption a significant conclusion can be drawn from the fact that the dibasic lactone acid ($\text{C}_{22}\text{H}_{32}\text{O}_8$) is converted on distillation with acetic anhydride into a keto lactone with loss of carbon dioxide. The keto lactone yields a dibasic lactone-acid on oxidation, and the changes are entirely comparable with the corresponding degradation of ring A in the bile acid series. Interpreted in the same way in terms of the Blanc rule, the evidence indicates that the hydroxylated nucleus of gitogenin is a terminal, six-membered ring containing the grouping $-\text{CH}_2\text{CHOHC HOH}-$, as in VII. The observations could be explained equally well by placing the hydroxyl groups in the two β -positions, as in the alternate formula (IV, above) for digitogenin, but evidence reported in recent work by Jacobs and Simpson²⁷ argues against such a structure and supports formula VII. These authors observed that the dimethyl ester of gitogenic acid can be hydrolyzed to an acid ester, the second ester group being comparatively resistant to hydrolysis. Formula VIII, representing gitogenic acid as having one primary and one secondary acidic group, is in good agreement with this observation.

The early characterization of the other sapogenins was less extensive, but the observations were such as to indicate that in every case two oxygen atoms are bound in stable oxidic linkages. Tigogenin and sarsasapogenin are isomeric substances and they both contain one secondary hydroxyl group (acetylation, oxidation). In the case of chlorogenin two such groups are present and the substance appears to be isomeric with gitogenin but not identical with this substance.

Conversion to Sterol Degradation Products. Although the neutral sapogenins were at first regarded as C_{26} -compounds while cholesterol is a C_{27} -alcohol, it was long suspected that some relationship exists between the two groups of natural products. The first definite indication in this direction was from an observation of Ruzicka and van Veen²⁸ in 1929. In the course of Ruzicka's studies of the dehydrogenation of acidic sapogenins and other triterpenoids, sarsasapogenin was submitted to dehydrogenation

²⁶ Jacobs and J. C. E. Simpson, *J. Biol. Chem.* 110, 429 (1935)

²⁸ Ruzicka and van Veen, *Z. physiol. Chem.*, 184, 69 (1929)

with selenium, and there was isolated a fragrant, volatile substance, $C_8H_{16}O$, which was regarded as identical with methyl isohexylketone on the basis of a comparison of the semicarbazones. Since this ketone, $CH_3COCH_2CH_2CH_2CH(CH_3)_2$, had been obtained by Windaus as an oxidation product of cholesterol, the observation pointed strongly to the presence in the sapogenin of a side chain of structure identical with that of cholesterol. In 1934 Jacobs and Simpson²⁹ reinvestigated the dehydrogenation of sarsasapogenin and also obtained from the volatile fraction a ketone $C_8H_{16}O$. The semicarbazone melted several degrees lower than Ruzicka's preparation and it was regarded as different from the semicarbazone of synthetic methyl isohexylketone.³⁰ The identity of the substance is still in doubt. The same ketone was obtained in a similar manner from gitogenin. A cleavage of the side chain under milder conditions was achieved by heating either sarsasapogenin or gitogenin with a mixture of hydrochloric and acetic acids, and in this case the volatile product was an unsaturated carbonyl compound, $C_8H_{14}O_3$. The substance appears to be either an unsaturated dihydroxyketone or a hydroxy-1,3-diketone (as enol). While the identity of these cleavage products is still unsettled, the available results all point to the presence in the sapogenins of a C_4 -side chain which probably contains two oxide bridges.

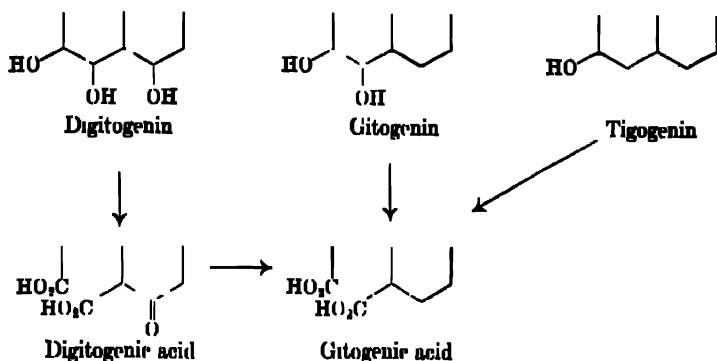
In the work just cited Jacobs and Simpson made the important discovery that the mixtures resulting from the dehydrogenation of either sarsasapogenin or gitogenin contain the Diels hydrocarbon, methyleyclopentenophenanthrene. The ring system of the sapogenins must correspond with that of the sterols! The formation of the Diels hydrocarbon as a degradation of other natural products, for which the presence of the aetioholane ring system is established by independent evidence, leaves little doubt on this score.

The exact relationship between digitogenin and gitogenin was established by Tschesche³¹ in 1935 (June). Digitogenin on mild oxidation yields a dibasic keto acid, while in gitogenic acid, the oxidation product of gitogenin, the carbonyl group is lacking. Tschesche succeeded in reducing digitogenic acid by the Wolff-Kishner method and found the product to be identical with gitogenic acid. He also succeeded in correlating the third sapogenin from *D. purpurea*, for tigogenin was found to yield gitogenic acid on oxidation. The relationships are expressed in the accompanying formulas.

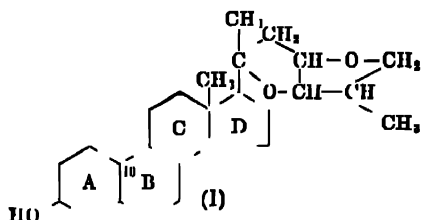
²⁹ Jacobs and J. C. E. Simpson, *J. Biol. Chem.*, **105**, 501 (1934).

³⁰ *Ibid.*, *J. Am. Chem. Soc.*, **56**, 1124 (1934).

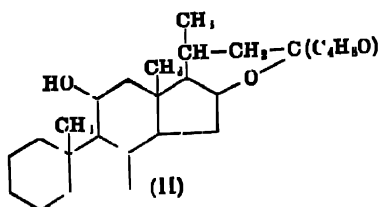
³¹ Tschesche, *Ber.*, **68**, 1090 (1935).



Tschesche discussed his results in terms of a provisional formulation of the sapogenins suggested by Windaus,³² according to which tigogenin, for example, was represented as in I. To reconcile his generally accepted



C_{26} -formulas for the sapogenins with the evidence indicating the presence of the cholane ring system and of the side chain structure of cholesterol, a C_{27} -compound, Windaus suggested that the usual C_{10} -methyl group of the sterols is in this case missing. This conception of the structure was not long sustained. In a paper published one month before Tschesche's report, Simpson and Jacobs³³ announced that added analytical data in the sarsasapogenin series had convinced them of the necessity of revising the formula of the genin to a C_{27} -basis. From a consideration of other observations which are most conveniently discussed in another connection, they suggested for sarsasapogenin the formulation II.



³² Windaus, *Nachr. Ges. Wiss. Göttingen*, 89 (1935).

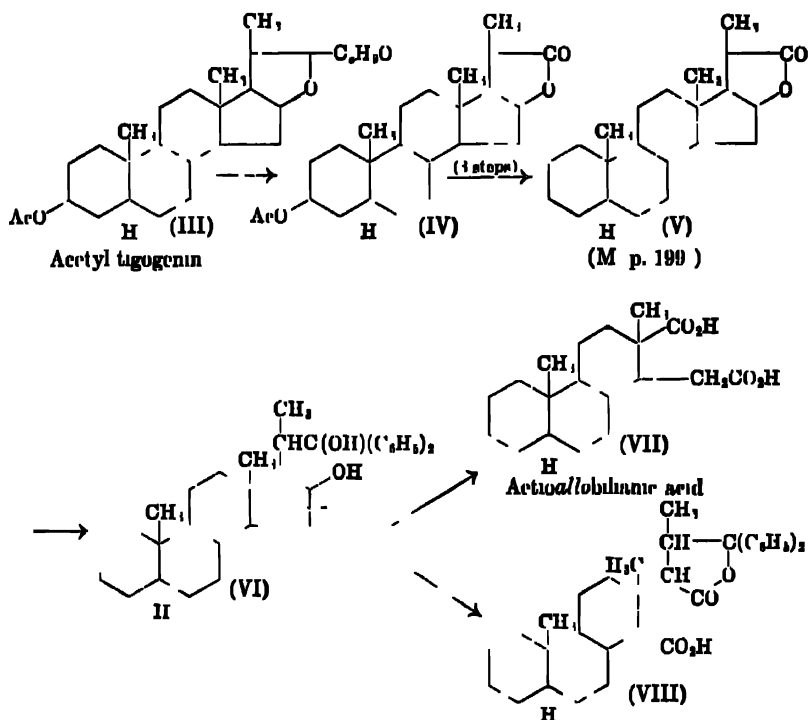
³³ J. C. E. Simpson and Jacobs, *J. Biol. Chem.*, 109, 373 (1935).

In July Tschesche and Hagedorn²⁴ achieved the long-sought objective of obtaining by nonpyrolytic methods a degradation product of known structure, for they succeeded in converting tigogenin into aetioallobilanic acid. In this substance three of the original rings are still intact and there are remnants of the fourth ring. The two angular methyl groups characteristic of the sterols are also present in the degradation product. The combined evidence from this observation, from the isolation of the Diels hydrocarbon, and from the isolation of C₉-ketones arising from the cleavage of the side chain, coupled with the evidence correlating tigogenin, digitogenin, and gitogenin, establishes the presence in these sapogenins of twenty-seven carbon atoms. This evidence appears more convincing than that of even the most refined and modern methods of microanalysis, for the differences in composition are of the same order of magnitude as the errors of analysis. Tschesche and Hagedorn recalculated the older analytical data and noted that they agree about as well with the C₂₇-formulas as with those previously accepted. Their own analyses agreed somewhat better with the new formulation than with the old, and they made one further observation which supports the new conception of the structure. If the Windaus formula (I) were correct it should be possible to aromatize ring B by partial dehydrogenation over platinum catalyst. Such a reaction could not be realized, presumably because of the presence of a methyl group at C₁₀. It is interesting that the revision suggested by the workers in America in the case of sarsasapogenin was so promptly applied to the other genins by the German chemists on the basis of entirely different evidence.

In interpreting the degradation to aetioallobilanic acid, Tschesche and Hagedorn adopted for the side chain a type of structure somewhat different from that suggested by Simpson and Jacobs, acetyl tigogenin, the starting material, being represented as shown (in part) in formula III. The chief product of drastic oxidation with chromic anhydride was the acetyl derivative of a monobasic acid C₂₇H₄₂O₆, but there was also formed the acetyl derivative of a C₂₂-hydroxylactone to which the formula IV was assigned. The free hydroxylactone from IV was oxidized to a ketone and the latter was reduced by the Clemmensen method to the saturated lactone V. The lactone ring was found to be resistant to hydrolysis with cold alkali, but it could be opened by interaction with phenylmagnesium bromide, giving the diphenyl carbinol VI. In agreement with the formula, the substance contains two hydroxyl groups (Zerewitinoff determination). On treatment of VI with dehydrating agents, the tertiary hydroxyl group is not eliminated with the production of a double linkage, but instead water is lost between the two hydroxyl

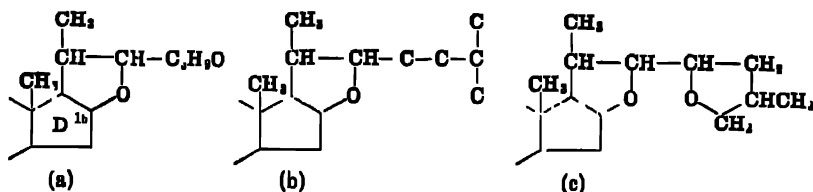
²⁴ Tschesche and Hagedorn, *Ber.*, 68, 1412 (1935).

groups with the formation of a compound having the properties of a tetrahydrofuran derivative. This indicates a 1:4 relationship between the two hydroxyl groups in accordance with the formulation. On oxidation of the diphenylcarbinol VI with chromic anhydride the five-membered

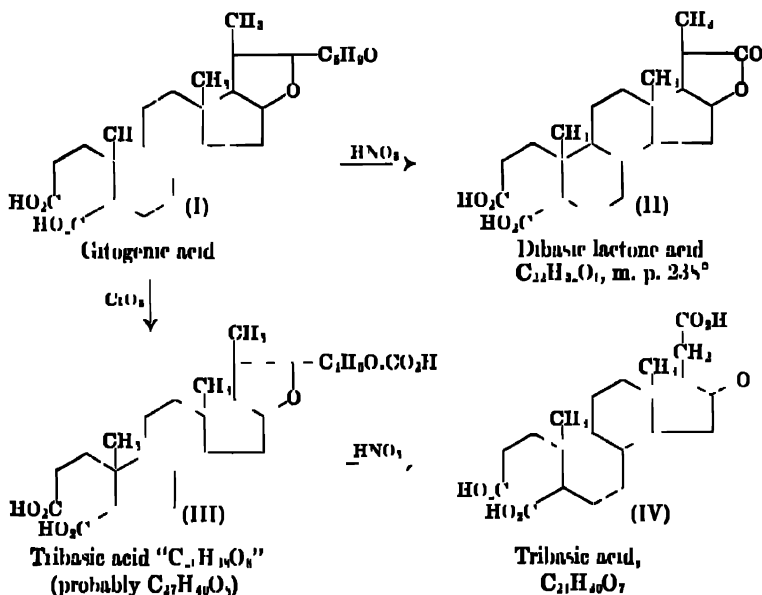


ring is cleaved in two directions as indicated by the dotted lines. One product was found to be identical with actioallobilanic acid (VII), while the other proved to be a monobasic lactone acid of composition and properties corresponding with the formula VIII.

The Structure of the Side Chain. The degradation provides good evidence of a part of the side chain structure and for the location of one of the oxidic oxygen atoms. It appears that a tetrahydrofuran ring is fused to the five-membered ring (D) of the actioallocholane system, that it carries a methyl group, and that the oxygen atom is joined at C₁₈ (structure a). There is little evidence as yet regarding the remaining part of the chain, except that the carbon skeleton probably conforms to the sterol pattern (b). Two of the five carbon atoms at the end of the chain must be connected by an oxide bridge, and Tschesche suggested as the most probable arrangement a methyltetrahydrofuran structure (c). In the



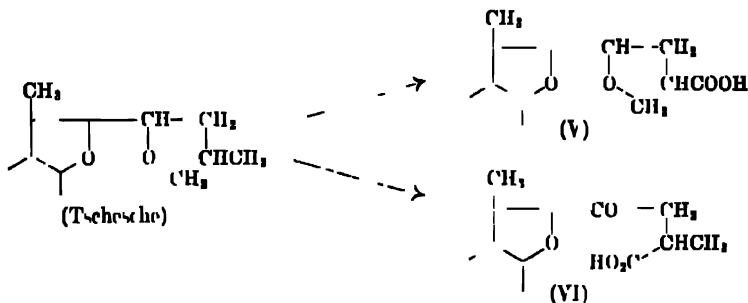
latest paper ¹⁵ on the subject this hypothesis has been retained, although it is recognized that there are difficulties in accounting for some of the earlier observations on this basis. In the extensive studies of Windaus a number of oxidation products were characterized very carefully, and in many cases the results are easily interpreted in terms of the newly established ring system. For example, the C_{22} -acid obtained by Windaus and Linsert ²⁶ by the oxidation of gitogenic acid (I) with nitric acid evidently has a structure (II) similar to that of the lactone obtained by Tschesche and Hagedorn.



Oxidations involving the extremity of the side chain are not as easily explained. After changes in the nucleus have been completed in the first stages of the oxidation of the sapogenins, chromic anhydride appears to exert a specific action in the part of the side chain included in the residue: $\text{C}_3\text{H}_5\text{O}$. Gitogenic acid is converted by the reagent into a tribasic acid which probably can be represented as in formula III. The original oxygen

²⁶ Tschesche and Hagedorn, *Ber.*, **68**, 2247 (1935)

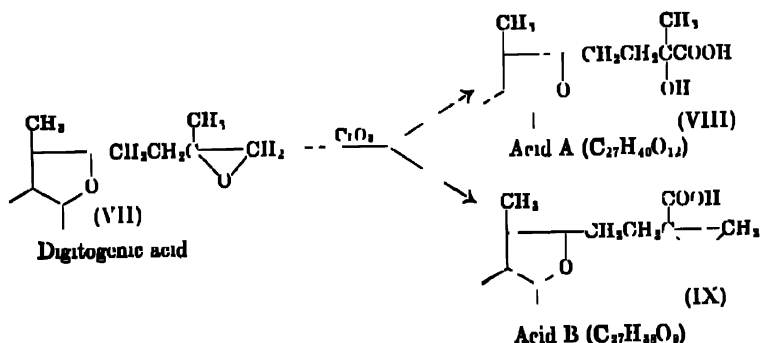
atom is retained and a methyl or methylene group has given place to a carboxyl group. A similar oxidation product was obtained by Windaus and Willerding²⁴ and by Windaus and Shah³⁰ by the oxidation of digitogenic acid with chromic anhydride in the cold: "tribasic acid B, $C_{26}H_{40}O_8$ "; probable formula: $C_{27}H_{48}O_9$. A further example of the same type of oxidation product is the monobasic acid $C_{27}H_{42}O_6$ obtained by Tschesche and Hagedorn^{34, 35} from acetyl tigogenin. The original acetyl group was hydrolyzed in the course of the purification and the substance can be represented by the formula $C_{21}H_{34}O(OH)C_4H_8O \cdot CO_2H$. The stoichiometric requirements of this evidently general mode of reaction would be satisfied by assuming that a terminal methyl group is oxidized without disturbance of the oxide bridge, as in formula V, and this formula-



tion would account also for the fact that the esters of the three acids in question do not react with carbonyl reagents and apparently contain no hydroxyl groups in the side chain. Tschesche has pointed out, however, that such a reaction is without parallel among sterol derivatives and that the hypothesis is inherently improbable. The explanation tentatively preferred by Tschesche is that the oxide ring opens with the formation of a γ -keto acid grouping (VI), but this view is hardly consistent with the observation that the esters of the acids fail to form oximes or semicarbazones or to absorb hydrogen in the presence of a catalyst. Since Tschesche and Hagedorn used the Fischer method for the esterification of the acid $C_{27}H_{42}O_6$, it might be argued that the substance formed is inert because it has the structure of a lactol ether rather than of an ester. The equally inert compounds of Windaus, however, are surely esters for they were prepared with the use of diazomethane. Another possible objection to the formulation is that if the product of the chromic anhydride oxidation of gitogenic acid (III, above) is a γ -keto acid it would be expected to yield the dibasic lactone acid II on further oxidation with nitric acid. Instead, it gives a C_{21} -tribasic acid, which possibly has the formula IV.

³⁰ Windaus and Shah, *Z physiol Chem*, 151, 86 (1926)

There is one observation from the early work which is particularly difficult to explain in terms of Tschesche's formulation of the side chain. On oxidizing digitogenic acid with chromic anhydride in a hot solution, Windaus and Willerding²⁴ obtained a pentabasic "acid A," to which reference already has been made. The substance easily loses carbon dioxide and gives a tetrabasic acid to which the formula " $C_{25}H_{35}O_{10}$ " originally was assigned. The analytical figures for both acids agree fairly well with the revised formulas having an additional methylene group. In characterizing the tetrabasic acid, probably $C_{25}H_{40}O_{10}$, it was found that the tetramethyl ester, prepared with diazomethane, liberates one mole of gas when treated with methylmagnesium iodide. Since four carboxyl groups and one original oxide ring account for all but one of the ten oxygen atoms, it is inferred that the oxygen atom present as a hydroxyl group corresponds to that of the second original oxirane bridge. The oxidation must involve the opening of an oxide ring with the liberation of a hydroxyl and a carboxyl group. Since a secondary hydroxyl group could hardly escape either oxidation or lactonization in the course of the reaction, it is inferred that the group in question is tertiary. It would seem, then, that the oxirane bridge probably connects a quaternary carbon atom and a terminal methylene group, and from these considerations it appears possible that the sapogenin side chain has the structure indicated in formula VII. This would account for the formation and the properties



of the pentabasic acid A (VIII), and perhaps also for the second type of chromic acid oxidation product, the tribasic acid B from digitogenic acid acquiring the formula IX. Windaus and Shah's²⁵ conversion of acid B by treatment with hydrogen iodide, followed by zinc dust, into a reduction product $C_{27}H_{46}O_8$, a reaction involving the selective opening of one of the two oxide rings, is likewise understandable.

In comparison with simple compounds containing ethylene oxide and α -oxido acid groups, the sapogenins and their oxidation products seem more stable than might be expected on the basis of the structures under

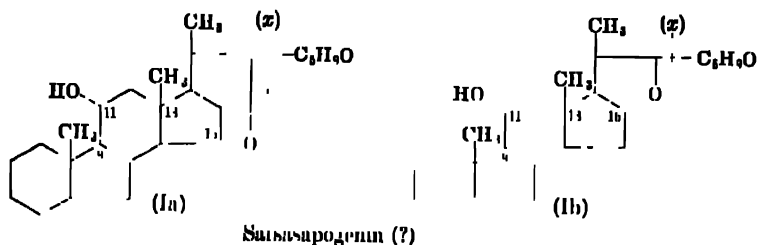
discussion. They are, however, quite comparable in this respect with another natural product which very probably contains an ethylene oxide group, namely, scymnol (page 128). Scymnol, which like the sapogenins probably is a sterol oxidation product, contains an oxidic ring which is indifferent to hydrogenating and reducing agents and to mild oxidizing agents. On treatment with chromic anhydride at 50°, scymnol is converted into a C₂₇-compound containing, according to the evidence, an α -oxido acid group. The analogy lends definite appeal to the present suggestion, but clearly the problem awaits further experimental inquiry.

Other Unsettled Problems. The exact formulation of several of the compounds isolated in the researches of Kiliani and of Windaus is still uncertain in many details. It appears that the sapogenins are susceptible to oxidation in different parts of the molecule, and in addition to the cases cited there are examples of the hydroxylation of a quaternary carbon atom in the course of some of the oxidations. Such a hydroxylation apparently is involved, along with the cleavage of ring B, in Kiliani's conversion of digitogenic acid into digitic acid, which probably is a keto-hydroxy tribasic acid, C₂₇H₄₀O₁₀, containing a tertiary hydroxyl group (formation of anhydrodigitic acid). The tribasic hydroxylactone acids C₂₂H₃₀O₁₀ and C₂₁H₃₀O₉, obtained by Windaus and Shah,⁵⁰ from digitoic acid and oxodigitogenic acid, respectively, probably belong in the same category, but in no case is the location of the tertiary hydroxyl group known.

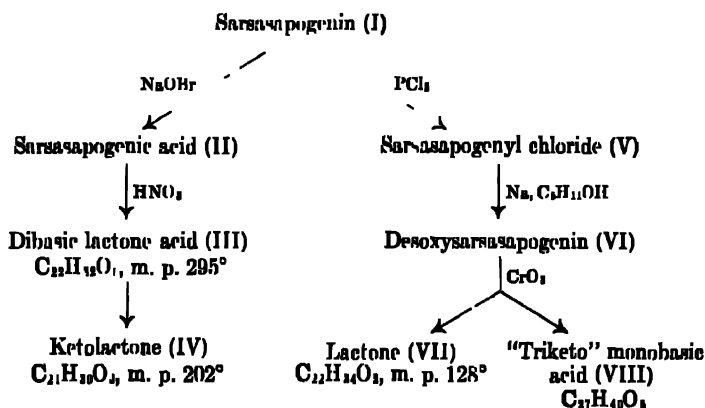
Unexplained is the observation of Windaus and Willerding²⁴ that the trimethyl ester of oxodigitogenic acid loses methyl alcohol on vacuum distillation with the formation of a compound (C₂₉H₄₂O₈) having the properties of an enol lactone. The reaction seems remarkable for an α -keto ester having a quaternary carbon atom in the β -position, as in the formula at present indicated for the ester, but it could be explained on the basis of the alternate structure corresponding to the location of the hydroxyl groups in ring A of digitogenin at positions 2 and 4. It will be recalled that the stability of digitogenic acid to alkalis also renders the 3,4-dihydroxy structure subject to some question, although the structure is supported by other apparently valid evidence. Another point of inconsistency was noted by Tschesche and Hagedorn.³⁵ These investigators found that tigogenin is precipitated nearly quantitatively by digitonin. (Tigogenin and digitogenin are not precipitated by the reagent, but neither is cholestanetriol-3,6,7, a compound of the β -type.) Since only positions 3 and 4 come into question as possible locations for the hydroxyl group, Tschesche and Hagedorn made comparison tests with cholestanol-4 and its epimer and found that neither substance forms a digitonide. The observations afford good evidence that the hydroxyl group of tigogenin

is located at C₃. Consequently there is no possibility of an inversion at C₃ in the degradation of tigogenin through tigogenone to aetioallobilanic acid, and tigogenin evidently belongs to the cholestane series (A/B:trans). In analogy with dihydrocholesterol, the oxidative fission of ring A would be expected to occur between positions 2 and 3, and not at C₃-C₄ as pictured above. Although the balance of evidence at present favors placing one of the hydroxyl groups of digitogenin and of gitogenin at C₄ rather than at C₂, the matter still awaits unambiguous decision.

Sarsasapogenin. Although this aglycone has not been correlated with the other members of the series except by the formation of common dehydrogenation products, the general similarity in properties and reactions is such as to indicate a close structural relationship. This has been clearly demonstrated in the recent degradation studies of Simpson and Jacobs.³⁷ These investigators interpret their observations in terms of the provisional formula Ia for sarsasapogenin, but a formulation (Ib) similar to that employed by Tschesche also appears admissible. Since further work may be expected to settle points of uncertainty in the near



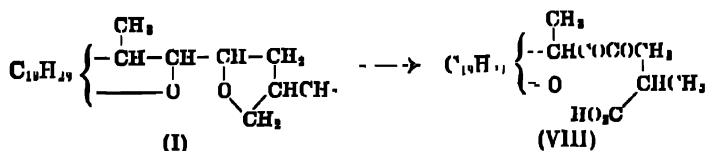
future, the present status of the problem will be indicated very briefly. The main degradations may be summarized as follows:



³⁷ J. C. E. Simpson and Jacobs, *J. Biol. Chem.*, 109, 573 (1935); 110, 505 (1935).

Hypobromite opens the hydroxylated ring giving the dibasic acid (II). From hydrolysis experiments with the dimethyl ester it appears that one of the carboxyl groups of sarsasapogenic acid is present in tertiary combination (as at C₁₃) and that the other is a secondarily bound group (as at C₉). The only position for the original hydroxyl group capable of satisfying these requirements is at C₁₁. Further oxidation of II gives (by cleavage at α in the formulas) a dibasic lactone acid (III) which gives a ketone (IV) on pyrolysis. Simpson and Jacobs consider that the lactone ring of III extends between the acidic group at C₁₁ and the hydroxyl group at C₁₅, and that a cyclobutanone ring is produced on pyrolysis. While the closing of a cyclobutane ring in the pyrolytic reaction would represent a most unusual reaction, Simpson and Jacobs prefer this interpretation to the alternate view that an acid with carboxyl groups attached to rings B and D yields a ketone, because thilobilianic acid, contrary to the Blanc rule, gives an anhydride on pyrolysis. In view of Vocke's work on the hexahydrodiphenic acids (page 151), the objection does not appear valid. It seems quite possible that the dibasic lactone acid III has a structure similar to the isomeric acid obtained from tigogenin (formula II, page 332), which, however, results from the opening of ring A.

The degradation of sarsasapogenin through the chloride (V) and the desoxy compound VI gave, as one product, a saturated lactone (VII). On the basis of formula Ib for sarsasapogenin, the lactone should have the same structure as Tschersche's lactone (formula V, page 331) from tigogenin, but the two substances differ considerably in melting point. It is possible that the two lactones are structural isomers (according to formula Ia for the sapogenin) or that sarsasapogenin and tigogenin differ in the configuration of the ring system. The second oxidation product (VIII) of desoxy-sarsasapogenin is regarded by Simpson and Jacobs as arising as follows:

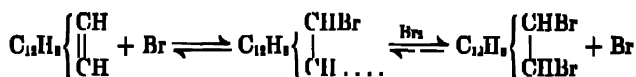


Only two of the carbonyl groups indicated in the formula could be detected by semicarbazone formation, however, and the acid appears to be similar to the products of chromic acid oxidation obtained in the other series. Until a clear differentiation can be made between the various possibilities the question of the structure of this part of the sapogenin molecule must remain open.

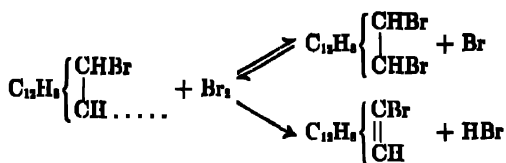
APPENDIX

Chapter I

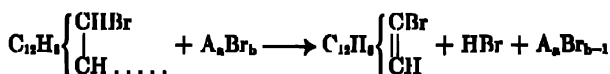
Reaction of Phenanthrene with Bromine (pp. 6-7). From a study of the kinetics of the formation of phenanthrene dibromide, Price, *J. Am. Chem. Soc.*, **58**, 1834 (1936), concluded that the reaction proceeds by a chain mechanism (even in the dark), the chain being propagated by bromine atoms and free radicals:



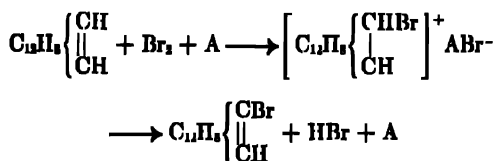
This conclusion was suggested by the observation that the reaction is inhibited by substances such as diphenylamine and tetrabromohydroquinone which can donate atoms of hydrogen to combine with bromine atoms and break the chain, while the inhibitors thereby revert to stable compounds. It is estimated that at 25° the chain consists of about 2,000 molecules, but that the chain length decreases with increasing temperature, accounting for the absence of a temperature coefficient in the measured reaction. Price, *ibid.*, **58**, 2101 (1936), also made the interesting observation that iodine, a typical catalyst for the halogenation of the benzene ring, inhibits the formation of phenanthrene dibromide, probably by interacting with chain-propagating bromide atoms. Furthermore, although iodine catalyzes the formation of hydrogen bromide in a solution containing phenanthrene, bromine, and the dibromide, it appears that the hydrogen bromide does not arise directly from the decomposition of the dibromide, as pictured in the classical addition-elimination theory. On adding iodine to a solution of phenanthrene dibromide in carbon tetrachloride (25°), no hydrogen bromide (or bromine) is produced until a trace of bromine is added, and even then the rate of HBr-formation is no greater than in a solution containing initially equivalent concentrations of phenanthrene and bromine. The addition-elimination theory seems definitely excluded by these observations, and it appears that the addition compound is formed, not as a necessary precursor of 9-bromophenanthrene, but from a radical, or other intermediate, common to both addition and substitution, the course of the reaction depending on the presence or absence of catalysts.



If a radical is involved in the first phase of the reactions, a catalyst A may function in combination with bromine as a hydrogen acceptor and so influence the rate of substitution.



As Price notes, it is equally possible that the essential intermediate is a coördinative complex of the type suggested by Pfeiffer and Wizinger, *Ann.*, 461, 132 (1928).



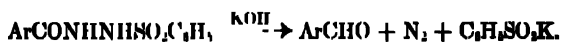
The effect of substituents on the phenanthrene-bromine equilibrium was investigated by Fieser and Price, *ibid.*, 58, 1838 (1936), who found that carboxylic ester groups and halogen atoms in the 2- and 3-positions decrease the free energy of the 9,10-addition of bromine ($-\Delta F_{27}^\circ = 3220$ cal., for phenanthrene), while the *ter*-butyl group has the opposite effect. The results parallel those obtained in the study of the free energy of oxidation of 9,10-phenanthrenhydroquinones by oxidation-reduction potentials (Ref. 54, p. 14), and there is a correlation between the observed effects of the groups concerned and their influence in retarding or facilitating substitutions in the benzene ring [L. F. Fieser and M. Fieser, *ibid.*, 57, 491 (1935)].

Friedel and Crafts Reaction (pp. 9-10). The condensation with propionyl chloride in nitrobenzene solution proceeds as in the other cases. Bachmann and Struve, *J. Am. Chem. Soc.*, 58, 1659 (1936).

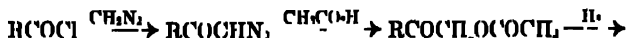
Phenanthraldehydes (pp. 10-11). Details of the preparation of 1-phenanthraldehyde⁴³ by the Sonn and Müller reaction are given by Bachmann and Boatner, *J. Am. Chem. Soc.*, 58, 2097 (1936). Hinkel, Ayling and Beynon, *J. Chem. Soc.*, 339 (1936), obtained the 9-aldehyde in 44% yield by the condensation of the hydrocarbon with hydrogen cyanide in chlorobenzene solution by means of aluminum chloride. The

use of chloromethyleneformamidine in similar syntheses in place of hydrogen cyanide also was investigated.

Considerable attention has been given to the development of general methods for the synthesis of aromatic aldehydes without specific reference to phenanthrene chemistry. F. E. King, L'Ecuyer and Openshaw, *ibid.*, 352 (1936) studied various applications of the methods of Sonn and E. Müller [*Ber.*, 52, 1927 (1919); 58, 1096 (1925)] and of Stephen [*J. Chem. Soc.*, 127, 1874 (1925)]. The latter synthesis consists in the reduction of a nitrile (as the imido chloride) with anhydrous stannous chloride in ether saturated with hydrogen chloride, and hydrolysis of the resulting alkimine complex: $RC\equiv N \longrightarrow RC\equiv N(HCl) \longrightarrow (RCH=NH, HCl)_2SnCl_4 \longrightarrow RCHO$. Wittig and Kethur, *Ber.*, 69, 2078 (1936), found that certain reductions with anhydrous stannous chloride proceed well in dioxane solution. Maxim and Mavrodineanu, *Bull. soc. chim.*, [5], 3, 1084 (1936) studied the Bouveault synthesis [Bouveault, *Compt. rend.*, 137, 987 (1903); Houben and Doeschner, *Ber.*, 43, 3435 (1910)]. In the typical case a Grignard reagent is condensed with N-formanilide and the aldehyde is obtained by hydrolysis of the product: $ArMgBr + OCH_3 \cdot N(CH_3)C_6H_5 \longrightarrow ArCH(OMgBr)N(CH_3)C_6H_5 \longrightarrow ArCHO + BrMgN(CH_3)C_6H_5$. An entirely new method, reported by McFadyen and Stevens, *J. Chem. Soc.*, 584 (1936), consists in the alkaline hydrolysis of a benzenesulfonacetylhydrazide, the steps in the preparation and decomposition of the intermediate being as follows:



The application is limited to the aromatic series. Another new method of transforming carboxylic acids into aldehydes, particularly useful in the case of aldehydes of a sensitive nature, is that of C. Grundmann, *Ann.*, 524, 31 (1936). The method involves the following steps:



9-Phenanthrol (p. 11). Fieser, Jacobsen and Price, *J. Am. Chem. Soc.*, 58, 2163 (1936), found that on treatment with bromine in methanol solution, phenanthrene is converted into an unstable complex consisting apparently of one molecule each of phenanthrene methoxybromide and phenanthrene dibromide. The complex yields 9-methoxyphenanthrene

and phenanthrene when warmed with alcoholic potassium hydroxide-acetate, and 9-phenanthrol is easily obtained from the mixture in 28-30% yield, based on the phenanthrene consumed. The synthetic preparation of 9-phenanthrol is discussed on page 356.

Phenanthrylamines (addition to p. 12). A very satisfactory method of preparing the 2- and 3-amino derivatives of phenanthrene was discovered by Bachmann and Boatner, *J Am Chem Soc*, **58**, 857, 2097 (1936), and employed in independent work by Mosettig and J. W. Krueger, *ibid.*, **58**, 1311 (1936) and by Fieser and Price, *ibid.*, **58**, 1838 (1936). The oximes of 2- and 3-acetylphenanthrene, prepared advantageously in pyridine solution, are submitted to the Beckmann rearrangement and the resulting acetylaminophenanthrenes are hydrolyzed to the amines. Bachmann and Boatner found that the rearrangements with phosphorus pentachloride proceed smoothly in benzene solution and that only traces of the alternate products, $C_{14}H_9CONHCH_3$, are formed. They prepared the new 1-phenanthrylamine by this method, the 1-acetylphenanthrene required being synthesized for the purpose from both 1-phenanthroic acid and 1-phenanthraldehyde. Details of the preparation of 1-phenanthroic acid from 1-benzoylphenanthrene are included in the paper, the process consisting in the Beckmann rearrangement of the oxime and the acid hydrolysis of the anhydride at 200° (compare Ref. 33).

9-Phenanthrylamine was obtained by Bachmann and Boatner from the 9-acetyl derivative which, in turn, was prepared by the action of methylmagnesium iodide on 9-cyanophenanthrene. A shorter process consists in heating 9-phenanthrol with ammonium sulfite and ammonia, following the general procedure of Bucherer [Fieser, Jacobsen and Price, *ibid.*, **58**, 2163 (1936); compare Russ. Pat. 40,988 (1935)].

Phenanthryl Halides (addition to p. 12). Since 2- and 3-phenanthrylamine are now readily available through the acetylphenanthrenes, they form convenient starting materials for the preparation of the corresponding halides [Bachmann and Boatner, *J Am Chem Soc*, **58**, 857, 2194 (1936); see also Fieser and Price, *ibid.*, **58**, 1838 (1936)]. 1-Phenanthryl halides can be obtained similarly from the less accessible amine. Bachmann and Boatner (second paper) found it advantageous to effect the diazotizations by the procedure which de Milt and Van Zandt, *ibid.*, **58**, 2044 (1936), developed for use with weakly basic or insoluble amines. A solution of the amine in pyridine is added to a stirred solution of nitrosylsulfuric acid in 2:1 sulfuric acid at 0°. For the introduction of chlorine or bromine atoms, Schwechten's modification of the Sandmeyer reaction proved very satisfactory [Schwechten, *Ber.*, **65**, 1605 (1932)]. This involves the precipitation and thermal decomposition of a complex,

formed by interaction of the diazonium compound with mercuric halide and potassium halide.

9,10-Dihydrophenanthrene (p. 13). Further details of the preparation³⁴ of the hydrocarbon are reported by Burger and Mosettig, *J. Am. Chem. Soc.*, **58**, 1857 (1936).

Steric Hindrance (p. 13). Whereas the alkaline hydrolysis of 1',3'-diketo-1,2-cyclopentenophenanthrene proceeds in both possible directions, the cleavage of 1',3'-diketo-3,4-cyclopentenophenanthrene occurs exclusively adjacent to the less hindered carbonyl group at C₈, and results in the formation of pure 4-acetyl-3-phenanthroic acid. On decarboxylation this yields the new 4-acetylphenanthrene, L. F. Fieser, M. Fieser, and Hershberg, *J. Am. Chem. Soc.*, **58**, 2322 (1936).

Oxidation of Phenanthrenequinones (p. 15). In the oxidation of retenequinone (1-methyl-7-isopropylphenanthrenequinone) with hydrogen peroxide, a lactone similar to that described by Fieser²⁷ is formed as a by-product along with the corresponding diphenic acid. Adelson, Hasselstrom and Bogert, *J. Am. Chem. Soc.*, **58**, 871 (1936).

Structure of Pyrene (p. 17). A theoretical discussion of the problem, based upon spectroscopic evidence, is given by Clar, *Ber.*, **69**, 1671 (1936).

Substitution Reactions of Chrysene (p. 18). Funke and co-workers (Eugen Müller, Vadasz, and Ristic), *J. prakt. Chem.*, **144**, 242, 265 (1936); **145**, 309 (1936); **146**, 151 (1936), found that chrysene can be converted in good yield into a monobenzoyl derivative, while in the Friedel and Crafts reaction with acetyl chloride a similarly substituted acetyl compound is the chief product but is accompanied by an isomer. Dibenzoyl derivatives were obtained by direct substitution and through a dibromochrysene, and various transformations are reported. Structures are assigned to the new compounds, but the evidence is still incomplete.

Pschorr Synthesis (pp. 28-31). Sharp, *J. Chem. Soc.*, 1234 (1936), employed the Pschorr synthesis for the preparation of 9-acetylamino-2,3,4,6-tetramethoxyphenanthrene, a compound of interest in connection with the chemistry of colchicine (p. 38). Ruggli and Staub, *Helv. Chim. Acta*, **19**, 1288 (1936), have shown that the function of the carboxyl group in the general synthesis is in part to control the condensation in such a way that the stilbene derivative is in the steric condition suitable for subsequent cyclization.

Sinomenine (p. 37). Further studies are reported by Goto and co-workers (Shishido, Ogawa, Saito), *Bull. Chem. Soc. Japan*, **10**, 252, 481, 597 (1935).

References to the Work on Drug Addiction (pp. 45-48). Chemical investigations of alkaloids of the morphine group are reported by Small and coworkers (Lutz, Mosettig, F. L. Cohen, Faris, Yuen, Fitch, W. E.

Smith, Morris and Eilers) under the following headings: **desoxycodaine studies**, *J. Am. Chem. Soc.*, **53**, 2214, 2227 (1931); **54**, 793, 802 (1932); **56**, 1738 (1934); **reduction studies**, *ibid.*, **54**, 4715 (1932); **56**, 1741, 1928, 2466 (1934); **57**, 361, 364, 2651 (1935); **Grignard reaction**, *ibid.*, **58**, 192, 1457 (1936); **miscellaneous**, *ibid.*, **55**, 2874, 3863 (1933); **56**, 1930 (1934); *J. Org. Chem.*, **1**, 194 (1936). The synthetic work of Mosettig and co-workers (van de Kamp, Burger, J. W. Krueger, R. A. Robinson) is reported as follows: **phenanthrene derivatives**, *J. Am. Chem. Soc.*, **52**, 3704 (1930); **54**, 3328 (1932); **55**, 2981, 2995, 3442, 3448 (1933); **56**, 1745 (1934); **57**, 1107, 2189 (1935); **58**, 1311, 1568, 1570, 1857 (1936); **dibenzofuran derivatives**, *ibid.*, **57**, 902, 2186 (1935); **58**, 688 (1936).

Pharmacological studies of the **synthetic compounds** by Eddy and by R. G. Smith are reported in the series: *J. Pharmacol.*, **48**, 183 (1933); **51**, 75, **52**, 275 (1934); **54**, 87 (1935); **55**, 354, 419 (1935); **58**, 159 (1936). Pharmacological studies of the alkaloids by Eddy, Sinnon, Aherns, Reid, Howes, H. Krueger, Gay, Lampe, C. I. Wright, Barbour and Foster are reported thus: **general physiological actions**, *ibid.*, **45**, 339, 361 (1932); **49**, 319 (1933); **51**, 35 (1934); *Am. J. Phys.*, **47**, 597, 614 (1935); *J. Pharmacol.*, **52**, 468 (1934); **53**, 430 (1935); **55**, 127, 257 (1935); **56**, 421 (1936); **action on intestinal movement**, *ibid.*, **50**, 254, **51**, 85, 440 (1934); **55**, 288 (1935); **56**, 327 (1936); **respiratory effects**, *ibid.*, **51**, 327, 343 (1934); **53**, 34, **54**, 25 (1935); **56**, 39 (1936); **effect on blood pressure**, *ibid.*, **51**, 153, 170 (1934).

Chapter II

Chemistry of Retene (addition to p. 58). Studies of various derivatives and oxidation products of retene are reported by Bogert and his co-workers Hasselstrom and Adelson, *J. Am. Chem. Soc.*, **53**, 3462 (1931); **56**, 983 (1934); **57**, 1579 (1935); **58**, 653, 871, 2236 (1936). Nyman, *Ann Acad. Sci. Fennicae*, **A41**, No. 5, 80 (1934), prepared dihydroretene in good yield by reduction with sodium and amyl alcohol. The acetylation of the dihydro derivative was investigated and a number of new compounds described.

Primary Resin Acids (p. 68). As the result of further investigations, the previous views regarding the nature of the primary constituents of oleoresins and of their relationship to abietic acid have undergone revision. While it appeared from the work of Kraft⁵⁷ and of Hasselstrom and Bogert⁵⁸ that the crystalline acid mixtures obtained from fresh oleoresins contain only *d*-pimaric acid and levopimaric acid in varying pro-

portions, Kraft, *Ann.*, 524, 1 (1936), in a later investigation discovered a third acid called **pro-abietic acid** ($C_{20}H_{30}O_2$), m.p. 159-160°, $[\alpha]_D +11.5^\circ$, absorption maximum 243 m μ . This substance was isolated from the crystalline acids of *P. palustris* and from French galipot (*P. maritima*). With aqueous ammonia the acid mixture gives a crystalline precipitate of ammonium salts, from which pure *d*-pimaric acid and levopimaric acid can be obtained by fractional crystallization of the sodium salts. The new acid forms a very soluble ammonium salt and is found in the material precipitated from the ammoniacal mother liquor by neutralization with carbon dioxide. Pro-abietic acid is isomerized to abietic acid by boiling glacial acetic acid or by the action of alcoholic hydrochloric acid at room temperature. In the latter case the progress of the isomerization can be followed by the change in the optical rotation of the solution. From the character of the curve, in comparison with that for levopimaric acid, Kraft suggested that pro-abietic acid may be an intermediate product in the rearrangement of levopimaric acid to abietic acid. Although the acid is present in crystalline products obtained from fresh oleoresins under very mild conditions and without the use of acids, it may not be a true primary acid. The work of Wienhaus, Ritter and Sandermann, *Ber.*, 69, 2198 (1936), indicates that isomerizations may occur even in neutral solvents. They obtained abietic acid from *P. silvestris* without using acids but by repeated crystallization from methanol. *d*-Pimaric acid and levopimaric acid are possibly the only diterpene acids initially present in oleoresins. Probably all of the so-called primary acids described in the literature, other than the two substances mentioned above and Kraft's pro-abietic acid, are mixed crystals containing varying amounts of these three acids and possibly abietic acid.

Further information concerning the structures of the acids and the relationship between them was obtained by Bacon and Ruzicka, *Chemistry and Industry*, 55, 546 (1936), who made the significant observation that levopimaric acid reacts with maleic anhydride at room temperature and yields a product, m.p. 227°, identical with that resulting from the addition of the anhydride to abietic acid. As the latter reaction occurs only at a temperature above 100°, it is inferred that the isomerization of levopimaric acid to abietic acid is reversible and that the conversion of the more stable isomer into the addition product proceeds through an isomerization at the elevated temperature to levopimaric acid. The observation was confirmed in independent work by Wienhaus and Sandermann, *Ber.*, 69, 2202 (1936), who found also that levopimaric acid, but not abietic acid, reacts at room temperature with benzoquinone and with α -naphthoquinone to give beautifully crystalline, yellow products.

Since the other constituents of oleoresins do not form addition products under the same conditions, the reaction offers promise of providing a method for the quantitative determination of levopimaric acid in the resins.

Kraft's early suggestion⁵⁷ that levopimaric acid is a bicyclic compound containing three conjugated double bonds (p. 68) has not been substantiated. Bacon and Ruzicka found that, although the acid yields only a dihydro derivative when hydrogenated in neutral solvents in the presence of Adams' catalyst, dihydrolevopimaric acid rapidly absorbs one mole of hydrogen in acetic acid solution, using the same catalyst. From the mixture of stereoisomers produced there was isolated a tetrahydro derivative, m.p. 195-197°, which gives no coloration with tetranitromethane, indicating the absence of a double linkage. The molecular refraction of the methyl ester agrees with that calculated for a tricyclic system and the ester yields retene on dehydrogenation with palladium charcoal. The conclusion that levopimaric acid contains three rings and two double bonds was reached independently by Kraft, in the work cited. Ozonization of levopimaric acid in the cold gave no formaldehyde, contrary to the requirements of the early formulation, and dihydrolevopimaric acid absorbed but a single atom of oxygen on titration with perbenzoic acid. Titrations of levopimaric acid itself also indicated the presence of two double bonds. Kraft originally suggested that the acid contains a triene system because this seemed to account for the displacement of the ultraviolet absorption band in the direction of longer wave lengths, as compared with abietic acid, but he later recognized that the absorption maximum at 272.5 m μ is indicative, rather, of the presence of two conjugated double bonds in the same ring (see p. 374). In analogy with other cases, the absorption maximum of 237.5 m μ found for abietic acid indicates the presence in this acid of two conjugated double bonds distributed between two rings. In view of the evidence that one of the ethylenic linkages extends to the carbon atom (7) carrying the isopropyl group (p. 60), it is probable that the double bonds of abietic acid are located at the 7,8-, and 14,9-positions (for the numbering system, see p. 59). To account for the ready interconversion of abietic acid and levopimaric acid, it is suggested that the latter substance has the $\Delta 7,8;14,13$ -structure. The double bond of the maleic anhydride addition product is assumed to occupy the 8,14-position. This is consistent with the observation of Wienhaus and Sandermann (*loc. cit.*) that the addition product, as the methyl ester $C_{25}H_{34}O_5$, yields on ozonization a keto ester-acid $C_{25}H_{34}O_8$. This product must arise from the severing of a linkage extending to a bridge head, as at the 8,14-, 5,13-, or 9,14-position. The dihydro derivatives of abietic acid and of

levopimaric acid may be structural isomers, or they may differ in the configuration at C₇ or C₁₃. According to this view, pro-abietic acid is not an intermediate isomerization product but has the $\Delta^5,13;14,9$ -structure.

Clemmensen Reduction (p. 72). Evidence that the results are often improved by adding toluene to the reaction mixture is presented by Martin,⁷¹ *J. Am. Chem. Soc.*, **58**, 1438 (1936). The modification is particularly useful in the reduction of β -aroylpropionic acids and of certain methoxylated compounds. Fieser and Hershberg, *ibid.*, **58**, 2382 (1936), encountered a remarkable side reaction in the reduction of β -(1,5-dimethoxy-4-naphthoyl)-propionic acid. In the formation of the abnormal product the ketonic group is reduced as usual, but the aromatic ring to which it is attached is hydrogenated in the course of the reaction and a methoxyl group in the para position is eliminated as well.

Friedel and Crafts Reaction with β -Naphthyl Methyl Ether (p. 74). Following the discovery by Haworth that β -naphthyl methyl ether is substituted largely in the 6-position when the Friedel and Crafts reaction with acid chlorides is conducted in nitrobenzene solution (p. 209), Short, Stromberg and Wilcs, *J. Chem. Soc.*, 319 (1936), found that this is true also in the reaction with succinic anhydride. They state that in nitrobenzene solution the 6- and 1-substitution products are formed approximately in the ratio 9:1, but no yields are recorded. On using carbon bisulfide as the solvent only the 1-substitution product was isolated (no yield given). Hill, Short and Higginbottom, *ibid.*, 317 (1936), found that when the 1-position is blocked by a methyl group substitution occurs almost entirely in the 6-position. Using nitrobenzene as the solvent they obtained β -(2-methoxy-1-methyl-6-naphthoyl)-propionic acid in 78% yield. The keto acids were employed for the synthesis of 7-methoxy- and 2-methoxy-1-methylphenanthrene.

Synthesis of Retenequinone (p. 75). By a process similar to that of Ruzicka and Waldmann,⁷⁵ Keimatsu, Ishiguro and Sumi, *J. Pharm. Soc. Japan*, **56**, 588 (1936), synthesized 9-methoxyretene; retenequinone was obtained on oxidation. As in the work of Ruzicka and Waldmann, the 7-isopropyl-1-naphthyl methyl ether required as starting material was synthesized from isopropylbenzene and succinic anhydride. The synthetic 9-methoxyretene, m.p. 108-108.5°, was identical with the methyl ether of a compound prepared by Fieser and M. N. Young, *J. Am. Chem. Soc.*, **53**, 4120 (1931), by the reduction of retenequinone with zinc dust and acetic acid, and regarded by them as 9-retenol on the basis of certain observations and inferences. The synthesis establishes the fact that it is the oxygen atom closest to the methyl group in retenequinone which is eliminated on reduction.

Perlman-Davidson-Bogert Synthesis (pp. 77-78). Shortly after the preliminary announcement by Bogert⁷⁸ in 1933 of the new phenanthrene synthesis, Cook and Hewett (p. 161) reported the independent discovery of the same method. In the hands of Cook and co-workers, and of other investigators, the general scheme of synthesis became an important tool in the solution of various problems, and the method was extended and applied in a number of cases (pp. 22, 164, 167, 210). Cook found that, in the cyclization of an unsaturated hydrocarbon of the type of II, the intramolecular addition of the aromatic nucleus to the double bond usually occurs to some extent in both possible directions, with the result that the normal reaction product is accompanied by an isomeric spiran (pp. 161-162). Eventually a satisfactory method of avoiding spiran formation and improving the yield was developed (Kon, p. 164; Cook, p. 162).

Information concerning the simplest application of the general synthesis has become available only recently. In preparing a quantity of *as*-octahydrophenanthrene (III), van de Kamp and Mosettig, *J. Am. Chem. Soc.*, **58**, 1062 (1936), isolated a small amount of a by-product which they regarded as a stereoisomeric hydrocarbon. From the results of an oxidation experiment, Cook, Hewett and Lawrence, *J. Chem. Soc.*, **71** (1936), suggested that the octahydrophenanthrene may be accompanied by a spirocyclic isomer, as in other cases. Finally Bogert and his collaborators reported the results of their study of the reaction [Bogert, *Science*, **84**, 44 (1936); Perlman, Davidson and Bogert, *J. Org. Chem.*, **1**, 288, 300 (1936)]. They were able to separate the by-product in a fairly satisfactory condition and to show that it is a spiran. The hydrocarbon was oxidized to α,α -pentamethylenehomophthalic acid, which was identified by comparison with a product obtained by the oxidation of a synthetic spiran of unequivocal structure.

The history of the discovery and exploitation of the important synthetic method is such as to suggest that it be referred to in the future as the Bogert-Cook synthesis.

Darzens Synthesis (p. 80). Darzens and Lévy, *Compt. rend.*, **202**, 73 (1936), found that α -chloromethyl derivatives of naphthalene and of α - and β -methylnaphthalene can be obtained in excellent yield from the hydrocarbon, trioxymethylene, and hydrogen chloride in glacial acetic acid solution. From α -chloromethylnaphthalene they obtained 1,9-dimethylphenanthrene by the standard synthesis (c) [*Idem, ibid.*, **202**, 427 (1936)].

Chapter III

1,2-Benzpyrene (p. 83, ff.). The pyrene numbering system used in this book (p. 16) is that originally employed by Cook, Hewett and Hieger,⁷ the discoverers of the carcinogenic constituent of coal tar. Some confusion has arisen in the literature because German authors have adhered to the numbering used in early literature and termed the hydrocarbon 3,4-benzpyrene. In the interests of uniformity, the English investigators very recently have reverted to the older system. The present author has agreed to use "3,4-benzpyrene" in future publications, but a change cannot be made in the book until the text is completely revised.

Carcinogenic Activity of Cholanthrene and 1,2-Benzanthracene Derivatives (pp. 88-93). The results of further tests conducted at the Royal Cancer Hospital, London, are reported in review papers by Cook, *Ber.*, 69A, 38 (1936), and by Cook, Haslewood, Hewett, Hieger, Kennaway and Mayneord, *Reports of the Second Congress of Scientific and Social Campaign against Cancer*, 1, 1 (1936). The results of studies of the carcinogenic action of the hydrocarbons synthesized at Harvard by L. F. Fieser, M. Fieser, Hershiberg, Newman and Seligman are reported in detail by Shear,^{10,17} *Am. J. Cancer*, 26, 322 (1936); 28, 334 (1936). Using the injection technique, and testing the malignant nature of the tissue by transplantation, Shear found that the average time of the appearance of tumors in pure-strain mice following the administration of 5-10 mg. of material affords an approximate relative measure of the potency of the compound. Relative carcinogenic activities determined in this way are as follows: methyleholanthrene, 2.5 mos.; cholanthrene, 3 mos.; 1,2-benzpyrene, 3.5 mos.; 1,2,5,6-dibenzanthracene, 7 mos. Qualitatively, at least, the results are in excellent agreement with those obtained at the Cancer Hospital. A comprehensive review of experiments with 1,2-benzpyrene and 1,2,5,6-dibenzanthracene in a number of other laboratories is given in the paper by Cook, *et al.* (*loc. cit.*)

Since methyleholanthrene and cholanthrene surpass in potency all of the other benzanthracene derivatives previously investigated, it was a matter of considerable interest to attempt to define the features of structure responsible for their striking activity. One aspect of the work in this direction conducted at Harvard was to investigate the effect of further elaborations of the methyleholanthrene molecule. Using the sterol numbering system (p. 111), this potent carcinogenic agent may be called 20-methyleholanthrene, and the introduction of a methyl group at the 16-position in the five-membered ring gives 16,20-dimethyleholan-

threne⁶⁵ (XIII, p. 107). Shear found that this modification of the molecule results in a distinct decrease in activity, the average time of appearance of tumors being 7.5 mos. 1',9-Methylene-1,2,5,6-dibenzanthracene⁶⁴ (from IX, p. 106) is another compound containing the skeletal structure of methylecholanthrene but having two additional carbon atoms incorporated in an aromatic ring, and it combines as well the features of structure of the carcinogenically active 1,2,5,6-dibenzanthracene. While the hydrocarbon has definite cancer-producing properties, the activity is considerably less than that of the dibenzanthracene (11 mos.). 15,16-Benzdehydrocholanthrene⁶³ (XVI, p. 107) has the characteristic cholanthrene ring system with a benzene ring fused to the five-membered ring. The substance shows only slight activity.

Although other variations in the same direction would be of interest, it seems evident from the present indications that an increase in the complexity of the structure results in a decrease in carcinogenic activity. The observation that cholanthrene is only slightly less potent than methylecholanthrene suggested that it might be profitable to investigate the further simplification of the molecule. Cholanthrene may be regarded as the 5,10-dimethylene derivative of 1,2-benzanthracene, but, in consideration of its high potency as compared with a number of methyl and dimethyl derivatives of the hydrocarbon, Cook *et al.* were led to conclude that "The essential structural feature is the pentacyclic system present in the molecule." In order to determine with certainty whether the special activity of cholanthrene is associated with the presence of the five-membered ring including carbon atoms 5 and 10, or merely with the presence of alkyl substituents at these positions, Fieser and Newman synthesized 5,10-dimethyl-1,2-benzanthracene (p. 353). The 5- and 10-monomethyl compounds were prepared for comparison. According to the latest (unpublished) observations of Shear, 5,10-dimethyl-1,2-benzanthracene produces transplantable tumors in mice and the tumors appear, on the average, after about three months. Further experiments may define more exactly the position of the compound in the series, but it is evident from the tests already completed that the hydrocarbon is comparable in carcinogenic activity with cholanthrene and methylecholanthrene. The five-membered ring characteristic of the cholanthrene system is of importance in contributing to the activity of hydrocarbons of this type only in that it includes carbon substituents at the 5- and 10-positions, and the intact ring is by no means essential. The results (Shear, unpublished) obtained with the monomethyl compounds are of further interest. It had been found by the research group in London that 5-methyl-1,2-benzanthracene is slowly, if definitely carcinogenic, and a similar conclusion was reached in experiments with material pre-

pared by a different synthesis. In marked contrast to the behavior of this isomer, 10-methyl-1,2-benzanthracene produced tumors in a large proportion of the mice tested in from three to four months. This compound of simple structure is nearly as active as the cholanthrenes.

When the previous results are viewed in retrospect, substitution at positions 5 and 6 appears to be of secondary importance. While it is true that 5,6-dialkyl-1,2-benzanthracenes regularly exhibit cancer-producing properties and that this fact provided a valuable clue which led to the discovery of carcinogenic activity among the cholanthrenes, the most important structural feature of methylcholanthrene is not the 5,6-substitution, or the presence of an added ring, but simply substitution at the meso-position 10. It is very interesting that 1,2-benzpyrene may be regarded as a 1,2-benzanthracene with a carbon substituent at the alternate meso-position 9, and that 8,9-dimethylene-1,2-benzanthracene,⁶⁸ an isomer of cholanthrene, shows some activity (Shear: tumors in 7 mos.). Obviously compounds with still simpler substituents at C₉ merit investigation. That too great an elaboration of an effective type of structure may result in loss of potency has been demonstrated repeatedly, and a further example is that the 7-methyl derivative⁶² of 8,9-dimethylene-1,2-benzanthracene is inactive. Particularly striking is the case of 10-isopropyl-1,2-benzanthracene, which was prepared by Cook⁸¹ in 1932. In contrast to the 10-methyl compound, the hydrocarbon was found to be devoid of carcinogenic properties. The substance is somewhat comparable with the rather slowly acting 16,20-dimethylcholanthrene, for this carries a branched-chain substituent at the same meso position.

The discovery of substances far simpler in structure than methylcholanthrene which approach it in carcinogenic potency has a possible bearing on the hypothesis that cancer-producing hydrocarbons may arise in the organism by the abnormal metabolism of cholesterol or of bile acids. Hitherto this hypothesis has found some support not only in the demonstration that bile acids can be transformed by chemical means into methylcholanthrene, but also in the apparently striking circumstance that this particular hydrocarbon, which carries as a mark of its possible origin the cyclopenteno ring characteristic of the sterols and sex hormones, is outstandingly potent in comparison with other compounds. While the possibility of the biological formation of methylcholanthrene remains undisputed, if, to be sure, quite unestablished, the observation that comparable activity is exhibited by simpler hydrocarbons lacking both the C₂₀-methyl group and the cyclopenteno ring characteristic of the sterols, and of a type not likely to arise in the process of metabolism,

weakens somewhat the circumstantial evidence favoring acceptance of the hypothesis.

Other Studies (pp. 92-93). The report of A. A. Morton, Clapp and Branch [Ref. 25 and *Am. J. Cancer*, 26, 754 (1936)] that triphenylbenzene and tetraphenylmethane produce malignant tumors in mice has not yet been confirmed in other laboratories. Shear (*loc. cit.* p. 349, and private communication) obtained entirely negative results with triphenylbenzene. Each of twenty mice received a subcutaneous injection of 10 mg. of a sample of the hydrocarbon which had been purified for an atomic weight determination. No tumors had appeared at the end of sixteen months and there were twelve survivors.

Negative results have been obtained with dehydronorcholene (Cook *et al.*, *loc. cit.*, p. 349, Shear), hexahydromethylcholanthrene (Shear), and dodecahydrocholanthrene (Cook, *et. al.*)

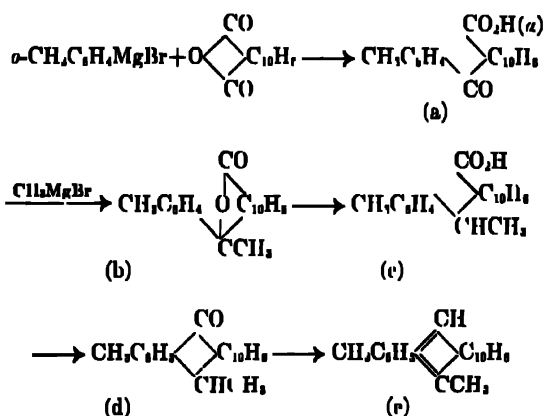
Tumors have been produced by the subcutaneous injection of aqueous solutions of the sodium salts of 1,2,5,6-dibenzanthracene-endo- α,β -succinic acid¹⁸ (Cook, *et. al.*) and of methylcholanthrene-choleic acid¹⁹ (Shear), in the latter case without any difficulty arising from the hemolytic action of uncombined desoxycholic acid. The actions of the former compound are described by Burrows and Cook, *Am. J. Cancer*, 27, 267 (1936), and by Parsons, *J. Path. Bact.*, 43, 1 (1936).

Reviewing the extensive literature of the biological studies of carcinogenic agents, Cook, *et al.*, note that "The results obtained in the last few years have shown that a variety of tumors (carcinoma of the skin, kidney, testis, bladder, liver, and uterus; sarcoma of the subcutaneous tissue, peritoneum and spleen) can be induced by pure chemical compounds." References may be found in this review to interesting studies of the rate of disappearance of carcinogenic hydrocarbons in the body, the effect of these compounds on such processes as respiration, the influence of genetic factors, the influence of the medium for the administration, the effect of inhibitors, the propagation of tumors by cell-free material, and other matters. A summary of the results of studies of the action of hormone preparations (pp. 217-219) shows that between thirty and forty instances of mammary cancer in male mice receiving oestrin have been recorded, but that no carcinogenic action truly comparable with that of the benzanthracene hydrocarbons has been observed.

The report of Browning and his collaborators²⁰ that the styryl quinoline derivative (styryl 430) has cancer-producing properties has been confirmed in further work by Browning, Gulbrandsen and Niven, *J. Path. Bact.*, 42, 155 (1936). The interesting discovery of Yoshida (see Cook, *et al.*, for references) that *o*-aminoazotoluene has carcinogenic properties has been confirmed in repeated experiments in various laboratories.

Administered by subcutaneous injection or orally, the substance produces tumors of the liver and bladder. Boyland and Brues (cited by Cook, *et al.*) found that 3,4,5,6-dibenzcarbazole produces proliferation of the bile ducts in a large proportion of cases when applied to the skin of mice. Since this substance may be regarded as a product of the dehydrogenation and condensation of two molecules of β -naphthylamine, the carcinogenic power of the compound is of interest in connection with the cases of bladder cancer found among workers engaged in the manufacture of dyestuff intermediates, particularly β -naphthylamine [Ref. 27, also W. C. Hueper, *J. Ind. Hyg.*, 18, 140 (1936)].

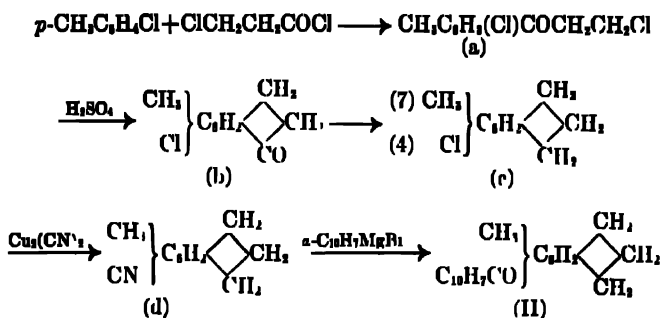
Synthesis of 5,10-Dimethyl-1,2-benzanthracene (addition to p. 94). For the preparation of this actively carcinogenic hydrocarbon and related compounds, Fieser and Newman, *J. Am. Chem. Soc.*, 58, 2376 (1936), developed a synthesis which combines various features of other methods. As in Cook's synthesis of the 6- and 7-isopropylbenzanthracenes (p. 94), the starting material was 1,2-naphthalic anhydride (previously called 1,2-naphthalene dicarboxylic acid anhydride), a compound which is now readily available by a convenient synthesis (Ref. 8, p. 221). On interaction of the anhydride with *o*-tolylmagnesium bromide, addition occurred



to some extent at each carbonyl group, but the desired *o*-toluyl- α -naphthoic acid (a) was the chief product. 1,2-Naphthalic anhydride is attacked chiefly at the less hindered β -group in both the Friedel and Crafts and the Grignard reactions, but it is only in the latter case that a substituent can be introduced at the ortho (or meta) position of the benzenoid ring. For the introduction of a methyl group, the ester of the keto acid (a) was treated with the Grignard reagent, as in analogous cases in the aliphatic series (p. 72, VII \longrightarrow VIII), but with only moderately satisfactory results. When the free acid (a) was treated with an

excess of methylmagnesium bromide, the lactone (b) was obtained in 86% yield. Reduction of the lactone was accomplished by the Clemmensen method and the acid (c) was cyclized with sulfuric acid to the anthrone (d). Reduction with zinc dust and alkali gave the desired 5,10-dimethyl-1,2-benzanthracene (e). 5-Methyl- and 10-methyl-1,2-benzanthracene were synthesized by obvious variations of the same scheme. 10-methyl-1,2-benzanthracene has been prepared also from 1,2-benz-10-anthrone and methylmagnesium iodide (Fieser and Hershberg, unpublished results). Some reduction occurred in the reaction of the keto acid (a) with Grignard reagents of higher molecular weight.

Synthesis of Methylcholanthrene (pp. 105-106). An improved process for the synthetic preparation of methylcholanthrene and related hydrocarbons has been described by Fieser and Seligman, *J. Am. Chem. Soc.*, **58**, 2482 (1936). 4-Chloro-7-methylhydrindene was employed in place of the 4-bromo compound (corresponding to I) for the preparation of the required ketone (II, p. 106). The hydrindene derivative was obtained by application of the reaction of F. Mayer and P. Müller, *Ber.*, **60**, 2278 (1927), rather than by the Blanc reaction and the malonic ester synthesis.⁸² *p*-Chlorotoluene was condensed with β -chloropropionyl



chloride by means of aluminum chloride, giving a mixture of the two possible chloroketones (a); cyclization to a hydrindone mixture (b) was accomplished by treatment with concentrated sulfuric acid. Reduction of the mixture afforded a single product, 4-chloro-7-methylhydrindene (c), which was converted into the corresponding nitrile (d) by interaction with cuprous cyanide in pyridine solution (modified Rosenmund-von Braun reaction, yield 78%). Condensation of the nitrile with α -naphthylmagnesium bromide gave the desired ketone II in 89% yield. It is interesting that the yield of the ketone is nearly twice as great as in the condensation of the Grignard reagent from 4-bromo-7-methylhydrindene with α -naphthoyl chloride⁸² or with α -naphthonitrile [Baehmann, *J. Org. Chem.*, **1**, 347 (1936)]. The pyrolysis of the ketone pro-

ceeded well on a large scale, giving pure methyleholanthrene in 49% yield. A by-product formed in the pyrolysis was characterized as a hydrocarbon ($C_{21}H_{20}$) and regarded as resulting from the reduction of the carbonyl group of the ketone II. The isolation of products of both reduction and oxidation (anthrones, see p. 108) in the Elbs condensation suggests that a process of disproportionation occurs as a side reaction. By the new synthesis methyleholanthrene can be prepared in quantity in over-all yield of 20% from *p*-chlorotoluene.

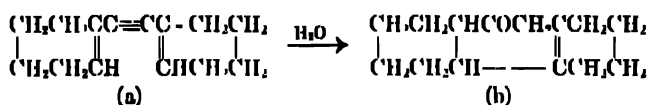
Bachmann (*loc. cit.*) reports that in the preparation of 1,2,5,6-dibenzanthracene by the Elbs reaction the yield in the pyrolysis was increased to about 50% by the addition of 10% of zinc dust. This modification does not appear to have been tried in the case of the methyleholanthrene synthesis. Bachmann prepared meso-dihydromethyleholanthrene through the disodium and dilithium compounds.

Synthesis of Phenanthrene and Hydrophenanthrene Derivatives (p. 110). The general interest in phenanthrene derivatives of various types has led to the development of a number of new methods of constructing the phenanthrene ring system. Certain of the methods are appropriately discussed in connection with special fields of investigation, but there are others which as yet have not found sufficient application to any one specific problem to be classified very satisfactorily. The two diene syntheses described on page 110 seem to belong to this class and it is perhaps appropriate to include, in a review of the extensions of these methods, references to certain other phenanthrene syntheses. (Another diene synthesis of polycyclic compounds is described on p. 388.

The diene synthesis of Fieser and Hershberg⁷³ proved very satisfactory for the preparation of 3-*ter*-butylphenanthrene [Fieser and Price, *J. Am. Chem. Soc.*, **58**, 1838 (1936)] and of chrysene and 2,3-dimethylchrysene, but attempts to prepare hydrocarbons of the 3,4-benzophenanthrene and 6,7-acchrysene series were successful only in the case of the dimethyl derivatives and then the yields were poor [L. F. Fieser, M. Fieser and Hershberg,⁷⁴ *ibid.*, **58**, 1463 (1936)]. Fieser and Holmes, *ibid.*, **58**, 2319 (1936), investigated a variation of the general plan of applying the Diels-Alder reaction to unsaturated cyclic compounds and found that α,β -unsaturated esters are capable of entering into the reaction. The addition of dienes to 3,4-dihydro-1-naphthoic ester occurs at a temperature somewhat higher than that required in the case of 3,4-dihydro-1,2-naphthalic anhydride (XI), affording hydrophenanthrene derivatives of a type hitherto inaccessible. Using the morphine numbering system because of the possible application of the results to morphine chemistry, the product obtained from butadiene is 5,8,9,10,13,14-hexahydrophenanthrene-13-carboxylic acid, a substance having a carbon sub-

stituent at the position which is indicated as being the most likely point of attachment of the ethanamine chain of morphine (p. 36). In order to provide a closer approach to the morphine type of structure a method was developed for the synthesis of substituted dihydronaphthoic esters. In a typical case anisole is condensed with succinic anhydride and the product is reduced and converted into α -oxalyl- γ -anisylbutyric ester. On hydrolysis with 15% sulfuric acid this is converted into α -keto- δ -anisylvaleric acid, which can be cyclized to 7-methoxy-3,4-dihydro-1-naphthoic acid with 65% sulfuric acid. By condensing the corresponding ester with butadiene, a 3-methoxyhexahydrophenanthrene-13-carboxylic acid was obtained. The methoxyl group occupies the same position as that of codeine (p. 25).

A novel synthesis of hydrophenanthrene derivatives is described by Pinkney, Nesty, Wiley and Marvel, *ibid.*, 58, 972 (1936). The dimagnesium halide of acetylene is condensed with two molecules of cyclohexanone and the resulting acetylenic glycol is dehydrated with 40% sulfuric acid to the diene (a). On treatment with 85% formic acid



this undergoes simultaneous hydration and cyclization and yields Δ^{11} -dodecahydrophenanthrene-9 (b). The fully saturated hydrocarbon obtained from this ketone was not dehydrogenated by selenium at 320°.

Another scheme for the synthesis of phenanthrenes involves the closing of the central nucleus through acetic derivatives of diphenyl or of a hydrodiphenyl. Chatterjee, *J. Indian Chem. Soc.*, 12, 591 (1935), prepared cyclohexanone-2-acetic ester from 2-carbethoxycyclohexanone by the Reformatsky reaction and hydrolysis, condensed the keto ester with phenylmagnesium bromide, and obtained diphenyl-2-acetic acid by treatment of the carbinol with sulfur, followed by hydrolysis. Cyclization with sulfuric acid gave 9-phenanthrol. Cook, Hewett and Lawrence, *J. Chem. Soc.*, 71 (1936), obtained 2-phenylcyclohexanol in almost quantitative yield by the condensation of cyclohexene oxide with phenyllithium. The alcohol was oxidized to 2-phenylcyclohexanone and the $\text{-CH}_2\text{CO}_2\text{H}$ group was introduced by the Reformatsky reaction, dehydration, and hydrogenation. Cyclization with sulfuric acid gave 1,2,3,4,9,10,11,12-octahydrophenanthrene-9, which yielded 9-phenanthrol on dehydrogenation with platinum. On converting 2-phenylcyclohexanol into an acetic acid derivative by the malonic ester synthesis and cyclizing the product, an isomeric octahydrophenanthrene was obtained. Sher-

wood, Short and Woodcock, *ibid.*, 322 (1936), synthesized 9-phenanthrol by a combination of the procedures of Chatterjee and of Cook and co-workers. Essentially the same method of synthesis, suitably elaborated, was employed by Hewett, *ibid.*, 596 (1936), for the synthesis of 3,4-benz-phenanthrene and its 2-methyl derivative.

Short, Stromberg and Wiles, *ibid.*, 319 (1936), have called attention to the interesting observation of Berger, *J. prakt. Chem.*, 133, 338 (1932), that phenanthrene can be obtained in fair yield by heating 2,2'-dimethyldiphenyl with sulfur at 250°. The English workers found that the carbino! from 2-methylcyclohexanone and *o*-tolylmagnesium iodide yields phenanthrene on similar treatment, but the reaction does not appear to be capable of general application.

Chapter IV

Occurrence of Cholesterol (p. 112). Although the cholesterol present in the brain and gall stones occurs almost exclusively in the free condition, the material found in most organs of the body is present partly as such and partly in the form of fatty acid esters. In a careful investigation of the cholesterol content of blood serum, which normally contains 0.15-0.25 g. of total cholesterol per 100 cc., Sperry, *J. Biol. Chem.*, 114, 125 (1936), found that the percentage of uncombined cholesterol is even more constant and lower than indicated in previous work. The average found for a number of different individuals was 26.9%, with a standard deviation from the mean of only $\pm 1.4\%$.

Cholesteryl Esters (p. 112). Nedswedski, *Z. physiol. Chem.*, 236, 69 (1935); 239, 165 (1936), observed the enzymatic synthesis of cholesteryl esters in colloidal solutions containing cholesterol, a higher fatty acid (palmitic, stearic, oleic), pancreatic lipase, and bile salts. Ester formation does not occur in the absence of bile salts, which appear to have a stabilizing influence on the enzyme. The salts of cholic, glycocholic, and taurocholic acid seem to be equally effective. The colloidal solutions of cholesterol were prepared by the usual process [Porgcs and Neubauer, *Biochem. Z.*, 7, 152 (1908)] of pouring an acetone solution of the sterol into water and evaporating the organic solvent.

Sitosterols (p. 112). Some further progress has been made in characterizing the phytosterol fractions which Anderson² designated α -, β -, and γ -sitosterol. From the observation that β -sitosterol and stigmasterol yield the same hydrogenation products, Bengtsson, *Z. physiol. Chem.*, 237, 46 (1935), concluded that the β -compound has the structure of 22-dihydro-

stigmasterol. This structure is also suggested for "sitosterol" by Vangelovici and Angelescu, *Bul. soc. chim. România*, **17**, 177 (1935), who identified acetone as one product of the oxidation of the acetate, indicating that the side chain terminates in $-\text{CH}(\text{CH}_3)_2$. The most soluble phytosterol fraction from wheat-germ oil, obtained by Anderson only in an impure condition and referred to by him as α -sitosterol, has been investigated further by Wallis and Fernholz, *J. Am. Chem. Soc.*, **58**, 2446 (1936), who succeeded in isolating two new doubly unsaturated sterols in a pure condition. One of these, α_1 -sitosterol, is an isomer of stigmasterol ($\text{C}_{28}\text{H}_{48}\text{O}$), while the other (α_2) probably is a homologue, $\text{C}_{30}\text{H}_{50}\text{O}$.

Preparation of Cholesterol (pp. 112-113). A rapid method of extracting cholesterol from brain tissue has been developed by Remesow and Lewaschowa, *Z. physiol. Chem.*, **241**, 81 (1936). After cooling the tissue with liquid air it is possible to grind the material to a fine powder without previous drying, and on extraction with acetone about 83% of the total cholesterol present can be obtained in a satisfactory condition.

Lanosterol (p. 113). In 1872 E. Schulze isolated from the wool fat of sheep a substance which, because it seemed to resemble cholesterol, was called "ischolesterol." Much later it was shown by Windaus and Tschesche, *Z. physiol. Chem.*, **190**, 51 (1930), that "ischolesterol" is a mixture of two substances, lanosterol and agnosterol. The formulas and properties found for these supposed sterols are listed in the table, p. 113. In a later investigation, H. Schulze, *ibid.*, **238**, 35 (1936), dehydrogenated a lanosterol-agnosterol mixture containing 92% of the former compound and identified 1,2,8-trimethylphenanthrene as a product of the selenium treatment. Since alkyl phenanthrenes have been obtained as degradation products of terpenoid compounds such as abietic acid (p. 58) and isoa-gathic acid (p. 69), but not of sterols, it appears from this evidence and from the empirical formula that lanosterol is a triterpene alcohol (pp. 318-321) and not a sterol. The compound is not altered by being heated with platinum at 300° and therefore all six-membered rings present in the molecule must be protected from ready aromatization by tertiary methyl groups. Previously polyterpenoid compounds had been known to occur only in plants, and it is of interest that the constituents of "ischolesterol" found in wool fat have been found incapable of absorption by the animal organism and consequently they cannot be acquired through foodstuffs. It therefore seems that the animal organism can synthesize polyterpenes as well as sterols. A further characterization of lanosterol has been undertaken by Dorée and Petrow, *J. Chem. Soc.*, 1562 (1936).

H. Schulze also investigated the substance **onocerin** from the root of *Ononis spinosa*, previously regarded as a sterol on the basis of a

rather fragmentary characterization. On dehydrogenation this was found to give in unusually good yield a hydrocarbon identified as 1,2,5,6-tetramethylnaphthalene, a characteristic degradation product of a number of triterpenoid compounds (p. 319). From the analysis of various derivatives, Schulze concluded that the formula of the substance is $C_{30}H_{50}O_2 \pm 2H$, and that it is in fact a triterpenoid, diatomic alcohol.

Sterols (p. 113). The isolation from wax of a substance named **bombicesterol**, $C_{27}H_{46}O$, m.p. 139-140°, $[\alpha]_D -31.5^\circ$, is described by Kawasaki, *J. Pharm. Soc. Japan*, **55**, 758 (1935).

Δ^5 -Cholestenone (p. 115). Butenandt and Schmidt-Thomé, *Ber.* **69**, 882 (1936), found that when the oxidation product of cholesterol dibromide is debrominated by short heating with zinc in neutral alcoholic solution the double bond does not migrate to a position of conjugation, and the product is Δ^5 -cholestenone (m.p. 127°), an isomer of the ordinary (Δ^4) cholestenone (m.p. 80°). Δ^5 -Cholestenone is stable in neutral solutions but rapidly rearranges into the Δ^4 -isomer in warm alcohol containing a trace of mineral acid or alkali, or in glacial acetic acid solution. When Δ^5 -cholestenone dibromide is debrominated with zinc and acetic acid, with sodium iodide and alcohol, or with zinc and weakly acidic alcohol, the conditions are such as to cause the unsaturated ketone first formed to rearrange in the course of the reaction.

Stereochemistry of the Steroids (addition to p. 117). Callow and F. G. Young, *Proc. Roy. Soc. (London)*, **A157**, 194 (1936), have made a preliminary study of the application of van't Hoff's principle of optical superposition to the sterols, bile acids, and other biologically important compounds containing the reduced cyclopentenophenanthrene ring system. The English investigators propose the term "steroid" as a generic name for the entire group of compounds having this common structural unit. On comparing the specific rotations of a number of diastereomeric pairs of compounds of opposite configuration at C_3 , it was found that in most cases the contribution of carbon atom 3 to the molecular rotation varies between fairly narrow limits, with a mean value of $\pm 11.5^\circ$. The effect of an inversion at C_7 is small and irregular. From a comparison of a number of pairs of compounds such as cholestanone and cholestenone it was established that a 4,5-ethylenic linkage generally causes a marked increase in dextrorotation (av. +220°). The effect of a 5,6-double bond is of about the same magnitude but in the opposite direction (av. -230°). Such simple empirical rules, if confirmed and extended, should provide a useful basis for the criticism of constitutional formulas suggested from chemical considerations.

Rosenheim Reaction (p. 118). According to observations of Schoenheimer and Evans, *J. Biol. Chem.*, **114**, 567 (1936), the Rosenheim color

reaction is given by sterols possessing a system of conjugated ethylenic linkages (e.g. ergosterol, scillaridin A) or by those capable of forming such a system by interaction with trichloroacetic acid (e.g. allocholesterol, see p. 361).

Microdetermination of Cholesterol (p. 119). Much attention has been given to the development of rapid and accurate methods for the determination of cholesterol in blood and other biological material. A particularly satisfactory procedure for the determination of free and total cholesterol is that of Schoenheimer and Sperry, *J. Biol. Chem.*, **106**, 745 (1934). Only 0.2 cc. of serum or whole blood is required and small amounts of cholesterol may be determined rapidly and accurately. The sample is extracted with acetone-alcohol and the free cholesterol is precipitated from a portion of the solution with digitonin. Total cholesterol is precipitated similarly from another portion of the extract after alkaline hydrolysis of the cholesteryl esters, and the cholesterol content of the two precipitates is determined by application of a color reaction with acetic anhydride and sulfuric acid, using a sensitive photometer. The accuracy is comparable with that obtainable with the macrogravimetric procedure of Windaus, and the introduction of colorimetry in the final step makes it possible to analyze a sample one one-hundredth the size previously required. The results may differ slightly in some cases because saturated sterols if present are precipitated by digitonin but do not give the color reaction. A modification of the Schoenheimer-Sperry procedure has been developed by C. Riegel and Rose, *ibid.*, **113**, 117 (1936), for the determination of 0.5 to 5 mg. of free and combined cholesterol in bile (1-10 cc.), with an accuracy of $\pm 5\%$. The initial extraction is made with ether.

In contrast to the colorimetric determination of digitonin precipitates, the direct application of colorimetry does not distinguish between free and combined cholesterol, since the esters give nearly the same color reactions as the sterol itself. Reinhold, *Am. J. Clin. Path.*, **6**, 22, 31 (1936), has shown, however, that by suitable modification of standard colorimetric methods it is possible to obtain results for total cholesterol which agree reasonably well with those of the gravimetric digitonin methods.

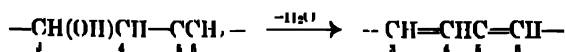
An entirely new method of determining both free and combined cholesterol has been introduced by Sobel, Drecker and Natelson, *J. Biol. Chem.*, **115**, 381, 391 (1936). Free cholesterol is precipitated as pyridine cholesteryl sulfate, unchanged esters and lipoids are extracted with petroleum ether, and the amount of cholesterol in the precipitate is estimated by the Liebermann-Burchard reaction. The values for total cholesterol in blood serum determined by the new method were compar-

able with those obtained by the cholesterol digitonide method, although they generally were slightly higher. The values found for free cholesterol, however, indicated that only 6-10% of the total sterol is present as such, whereas from the results obtained by the cholesterol digitonide methods it has been considered that the percentage is of the order 25-35% (see p. 357). The authors suggest that a part of the cholesterol formerly regarded as free may be present in some labile combination which is split by digitonin but not by the pyridine sulfate reagent.

Cholesterol Digitonide (p. 119). Dissociation into the components can be brought about by boiling a solution of cholesterol digitonide with alcoholic sodium acetate solution. On adding a suitable amount of ether the digitonin and the sodium acetate are precipitated while the cholesterol remains in solution. Lifschütz, *Biochem. Z.*, **282**, 441 (1935).

Isomers of Cholesterol (addition to p. 120). A number of observations have appeared in the literature regarding a substance known as "allocholesterol" (in p. 117°) which Windaus²⁹ prepared by the action of potassium acetate on cholesterol hydrochloride and which he regarded as the Δ^4 -isomer of cholesterol. It was thought that the substance rearranges easily under the influence of hydrochloric acid into cholesterol (as stated in the first edition, pp. 119, 281-282). Recently these conclusions have been found erroneous. Pure allocholesterol (Δ^4) was prepared for the first time by Schoenheimer and Evans, *J. Biol. Chem.*, **114**, 567 (1936), by application of the general method of Meerwein and R. Schmidt, *Ann.*, **444**, 221 (1925), for the reduction of α,β -unsaturated ketones to the corresponding unsaturated alcohols. Cholestenone, reduced at the carbonyl group with aluminum isopropylate, gave a mixture of the epimeric unsaturated alcohols, **allocholesterol** (m.p. 132°) and **epiallocholesterol** (m.p. 84°). The stereoisomers form a molecular compound which is not altered on repeated crystallization, but a separation can be accomplished by precipitating the allocholesterol with digitonin. The pure sterol recovered by the pyridine-ether method (p. 119) differed in properties from Windaus' "allocholesterol," and the latter substance was found on further investigation to be a mixture of allocholesterol and cholesterol. Pure allocholesterol does not rearrange to cholesterol in the presence of hydrochloric acid, but it does undergo dehydration to a hydrocarbon. The cholesterol obtained in the early experiments was that already present in the mixture, the dehydration of the isomeric sterol facilitating its isolation. In a later investigation Evans and Schoenheimer, *J. Biol. Chem.*, **115**, 17 (1936), showed that another supposed isomer known as " β -cholesterol" is in reality a molecular compound containing equal parts of dihydrocholesterol and **epiallocholesterol**. The former substance is removed by precipitation with digitonin.

Both allocholesterol and epiallocholesterol suffer dehydration with remarkable ease, the reactions proceeding nearly quantitatively on refluxing for two hours solutions of the sterols in 95% alcohol approximately *N*/30 in hydrogen chloride. The theoretical significance of the reaction is discussed on page 282, at least for the case of the *epi*-compound which alone was mentioned in the first account of the work. It now appears that the facile dehydration is characteristic of both α,β -unsaturated alcohols, regardless of the spatial arrangement of the hydroxyl group. The same substance, a doubly unsaturated hydrocarbon melting at 79° and giving a positive Rosenheim reaction, is formed in each case. Schoenheimer and Evans ascribe to the hydrocarbon the $\Delta^{3,4}$ -structure indicated in formula XIX, p. 282, but this appears inconsistent with the absorption spectrum of the compound, which shows maxima at 229, 235, and 240 $m\mu$. The selective absorption, like the color reaction, shows that the two double bonds are conjugated, but if they were contained in the same ring there should be a greater resemblance to the hydrocarbons ergostatriene ($\Delta^{5,7,22}$) and 7-dehydrocholestene, which show maxima at longer wavelengths (273, 280 $m\mu$). The spectrum of the hydrocarbon in question is indicative rather of a conjugated system distributed between two rings (see the work of Cullow, p. 374). Consequently it is suggested that the hydrocarbon has the $\Delta^{3,5}$ -structure and that it is formed by the 1,4-elimination of water from the unsaturated alcohol:



The hydrocarbon of Schoenheimer and Evans resembles cholesterylene, m.p. $78\text{--}79^\circ$, but it appears to be an isomer of this compound. Cholesterylene, was first described by Mauthner and Suida, *Monatsh*, 17, 29 (1896), and is prepared by dehydrating cholesterol with anhydrous copper sulfate at 200° or by heating cholesteryl chloride with quinoline. It shows selective absorption in the ultraviolet region with maxima at 294, 304, and 321 $m\mu$ [Heilbron, R. A. Morton and Sexton, *J. Chem. Soc.*, 47 (1928)]. In analogy with ergostatriene and 7-dehydrocholestene, it seems likely that the double bonds of cholesterylene are located in a single ring, possibly at the 2,3- and 4,5-positions. The dehydration at an elevated temperature would then involve the elimination of the hydroxyl group with a C_2 -hydrogen atom, and migration of the $\text{C}_7\text{--C}_8$ double linkage to a position of conjugation in ring A.

Epicholesterol is a second isomer of the natural sterol which has been characterized recently. The compound (m.p. 141°) was prepared by Marker, Oakwood and Crooks, *J. Am. Chem. Soc.*, 58, 481 (1936), by

passing oxygen into a solution of the Grignard reagent obtained from cholesteryl chloride. This gave a mixture of *epicholesterol* and cholesterol, from which the cholesterol was removed by precipitation with digitonin. For the preparation of the new sterol in quantity a partial separation is made by crystallization of the acetates, giving a product containing 80-90% of *epicholesterol*, and the remaining cholesterol is removed by crystallization of the benzoates [Marker, O. Kamm, Oakwood and Laucius, *ibid.*, 58, 1948 (1936)]. *Epicholesterol* was obtained in still another way by Marker, O. Kamm, Fleming, Popkin and Wittle, *J. Am. Chem. Soc.*, Paper X, in press. On oxidation with chromic anhydride, cholesteryl chloride gave 7-ketocholesteryl chloride, the latter was converted by potassium acetate in part into 7-keto*epicholesterol* as the acetate, and reduction by the Wolff-Kishner method afforded *epicholesterol*. Ruzicka and Goldberg, *Helv. Chim. Acta*, 19, 1407 (1936), found that *epicholesterol* is formed along with cholesterol on partially hydrogenating Δ^5 -cholestenone in the presence of Raney-nickel catalyst. A separation is accomplished as above, and the process affords a convenient method of preparing the epimer.

Marker and co-workers found that when *epicholesterol* is heated for sixteen hours in an alcoholic solution which is about 1 *N* in hydrogen chloride the substance is at least partially dehydrated to cholesterylene. The conditions are considerably more drastic than those under which the allo-compounds can be dehydrated, and it is questionable whether epimerization of the hydroxyl group has an appreciable influence on the ease of reaction.

Isomeric ethers and acetates of cholesterol have been investigated with interesting results. Stoll, *Z. physiol. Chem.*, 207, 147 (1932), observed that when cholesteryl *p*-toluene sulfonate is boiled with methanol it is converted into a methyl ether (m.p. 84°) which, since it is levorotatory like cholesterol, may be called the normal ether. Stoll discovered, however, that when the reaction is carried out in the presence of potassium acetate the product is an isomeric, dextrorotatory, abnormal ether, m.p. 79°. Other alcohols gave similar pairs of isomers. Similarly, Wagner-Jauregg and L. Werner, *ibid.*, 213, 119 (1932), found that cholesteryl chloride or bromide yields the normal ether when heated with methanol alone at 125°, but gives the abnormal ether in the presence of potassium acetate. They further found that the abnormal ether is converted into the normal compound when it is heated with hydrogen chloride and methanol, and they suggested that in the formation of ethers from cholesteryl halides or from the *p*-toluenesulfonate the abnormal ether is the primary product under all conditions and subsequently rearranges

to the normal ether under the influence of liberated acid unless this is neutralized by potassium acetate. While Stoll was of the opinion that the abnormal ether is an *epi*-compound, this conclusion seemed questionable to Wagner-Jauregg and Werner, for on hydrogenating the compound they found that although the reaction did not proceed smoothly, a small amount of normal dihydrocholesteryl methyl ether could be isolated.

More recently Beynon, Heilbron and Spring, *J. Chem. Soc.*, 907 (1936), studied the hydrolysis of the two series of cholesteryl ethers with the idea that the abnormal ethers might be derivatives of *epi*cholesterol and might provide a convenient route to this sterol. They found that whereas the normal ethers do not react with halogen acids in acetic acid solution at room temperature the abnormal ethers are converted easily under these conditions into the normal cholesteryl halide (chloride, bromide, or iodide). The English investigators provisionally regarded the isomers which are subject to this facile replacement of the alkoxyl group as ethers of *epi*cholesterol. The inference, however, is far from secure and it appears particularly questionable in the light of the observations of Wallis, Fernholz and Gephart, *J. Am. Chem. Soc.*, 59, 137 (1937), concerning an isomer of cholesteryl acetate which was obtained by the action of anhydrous potassium acetate on cholesteryl *p*-toluenesulfonate in acetic anhydride solution. Like the abnormal ethers, the new acetate is dextrorotatory, and on hydrolysis it yields an isomer of cholesterol, *i*-cholesterol (m.p. 74-75°), which is quite different from the epimer of the natural sterol and from the other two isomers described above. *i*-Cholesteryl acetate does not react with perbenzoic acid or with bromine and shows considerable stability to catalytic hydrogenation, although under special conditions of hydrogenation in glacial acetic acid with Adams' catalyst it yields the normal dihydrocholesteryl acetate. From these observations, Wallis, Fernholz and Gephart conclude that the acetolysis of cholesteryl *p*-toluenesulfonate is accompanied by a molecular rearrangement, and that a rearrangement in the reverse direction occurs in the hydrogenation reaction. They suggest that the rearrangement may be of the type commonly encountered in terpene chemistry and that *i*-cholesterol may contain, in place of a double linkage, a bridge bond extending from C₁ to C₇, with the hydroxyl group situated at C₆. Because of the evident analogies, they suggest that the abnormal ethers of Stoll may be of the same type.

Sterols of Lower Animals (pp. 121-122). A further indication that lower animal organisms utilize sterols derived from their food, rather than synthesize cholesterol, is furnished by the work of Klenk and Diebold, *Z. physiol. Chem.*, 236, 141 (1935), who isolated from sea anemone (*Anemonia sulcata*) a new sterol of the composition C₂₇H₄₄O. This

substance, to which the name **actiniasterol** has been given, is doubly unsaturated and possibly is a dehydrogenation product of cholesterol derived by the anemone (carniverous) from the food. The case is parallel to that of the doubly unsaturated oestriasterol, which appears to be produced in the oyster from a phytosterol of the diet.

Sterols in Bacteria (addition to p. 122). A number of attempts have been made to determine if sterols are present in bacteria or only in more highly organized forms of life, such as yeast, and the conclusion usually has been in the negative. Although Hercht, *Z. physiol. Chem.*, 231, 29, 279 (1935), reported the detection of sterols in tubercular bacilli, it appears from the observations of R. J. Anderson, Schoenheimer, Crowder and Stodola, *ibid.*, 237, 40 (1935), that all sources of contamination were not eliminated. In more recent work, however, Sifferd and R. J. Anderson, *ibid.*, 239, 270 (1936), isolated from the unsaponifiable portion of the fat of *Azotobacter chroococcum*, which had been cultivated on a scrupulously pure medium containing sterol-free glucose as the only organic constituent, a substance definitely characterized as a mixture of sterols (m.p. 156-158°). The substance constituted no less than 0.13% of the fat and the color reactions resembled those of cholesterol. The evidence clearly indicates that the organism in question is capable of effecting a sterol synthesis from sterol-free food.

Fucosterol (p. 122). Coffey, Heilbron and Spring, *J. Chem. Soc.*, 738 (1936), confirmed the identity of fucostanol and stigmastanol and established the presence of a double bond at C₇-C₈ in α -dihydrofucosterol, one of two dihydro derivatives obtained by the hydrogenation of fucosteryl acetate

Unsaturated Bile Acids (p. 124). The dehydration of most of the known hydroxycholanic acids theoretically can proceed in two directions, giving rise to a mixture of isomeric cholenic acids. Wieland, Kraus, Keller and Ottawa, *Z. physiol. Chem.*, 241, 47 (1936), found that mixtures indeed result on the thermal dehydration of the 3-, 6-, and 7-hydroxy acids, and they undertook the isolation of the components of these mixtures as pure individuals through the dibromides. In characterizing the products, use was made of the reaction with selenium dioxide, a reagent which, when shaken with a solution of a cholenic acid in chloroform and acetic anhydride, was found to bring about the transformation of $>C=CHCH_2-$ into $>C=CHCH(OH)-$. Apparently the reagent sometimes acts in a different manner when employed in aqueous alcoholic

solution (p. 373), for $\begin{array}{c} | \quad | \\ >CHC=CCH< \end{array}$ is converted into $\begin{array}{c} | \quad | \\ >C=C-C \\ =C< \end{array}$ in the aqueous solvent but remains unchanged when the experiment is conducted under anhydrous conditions (see also p. 369). In chloro-

form-acetic anhydride solution, the occurrence of a reaction was regarded as a good indication of the presence of an activated methylene group, but unfortunately the structure of the resulting hydroxy compound does not follow without question, for by an allylic shift the system $>C=CHCH(OH)-$ may change to $>C(OH)CH=CH-$ in the course of the reaction. Hydrogenation, resulting in the formation of cholanolic acid, allocholanolic acid, or both, afforded a means of recognizing a double bond extending to the 5-position.

From the results of the investigation, Wieland came to the following, necessarily qualified, conclusions. Lithocholic acid on dehydration yields chiefly Δ^3 -cholenic acid, along with some of the Δ^3 -isomer. The elimination of water from 6-hydroxyallocholanolic acid gave Δ^5 -cholenic acid as the chief product, along with some of the Δ^6 -acid, while 7-hydroxycholanolic acid yielded a mixture of Δ^6 - and Δ^7 -cholenic acids in which the former predominated. From desoxycholic acid there was obtained as the chief product a substance which probably is $\Delta^{2,11}$ -choladienic acid.

In the course of the work attempts were made to aromatize either ring A or ring B by the pyrolysis of the hydroxycholenic acids obtained in the oxidations with selenium dioxide. Theoretically such a reaction might occur, with the elimination of water and methane, but the results were entirely negative. The angular methyl group at C_{10} evidently renders the aromatization very difficult (see p. 254).

Determination of Bile Acids (p. 125). Doubilet, *J. Biol. Chem.*, 114, 289 (1936), developed methods for the analysis of bile for glyco- and tauro-bile acids, for total and conjugated bile acids, and for cholic and desoxycholic acids. In a typical case, bile from a human gall bladder was found to contain 7.6% of total bile acids, of which 3.3% was cholic acid and 4.3% desoxycholic acid. Of the total acids 20% was found to occur free, 46% was conjugated with glycine, and 34% was conjugated with taurine.

Ursodesoxycholic Acid (p. 126). Iwasaki, *Z. physiol. Chem.*, 244, 181 (1936), found the supposed "ursocholanolic acid"⁶³ to be identical with cholanolic acid and proved that ursodesoxycholic acid has the same structure as chenodesoxycholic acid and differs from this substance only in the configuration at C_7 .

Acids from Toad Bile (p. 128). Shimizu and Oda⁶⁰ oxidized trihydroxybufosterocholenic acid to a triketo acid and eliminated the oxygen atoms by reduction according to Clemmensen. The resulting bufosterocholenic acid formed a bromolactone on reaction with bromine, suggesting that the double bond is located in the side chain, probably in the β,γ - or γ,δ -position. Shimizu and Kazuno, *Z. physiol. Chem.*, 239, 74 (1936), carried the investigation a step further by oxidizing bufosterocholenic

acid with ozone or with chromic anhydride. The chief product of the reaction was identified as **bisnorcholanic acid** (p. 146), an observation which proves that the ring system is that of the other natural bile acids and that a double bond is located in the side chain at C₂₂-C₂₃, as in ergosterol. Shimizu and Kazuno suggest that the remainder of the side chain skeleton may also conform to that of ergosterol: $-\text{CH}(\text{CH}_3)\text{CH}=\text{CHCH}(\text{CH}_3)\text{CH}(\text{CH}_3)\text{CO}_2\text{H}$. The three hydroxyl groups of the acid from toad bile were shown to be located at positions 3, 7, and 12 by the ozone oxidation of the acid to a trihydroxybisnorcholanic acid identical with that obtained by the systematic degradation of cholic acid [*Idem*, *ibid.*, 244, 167 (1936)]

A second acid isolated by Shimizu and Kazuno, *ibid.*, 239, 67 (1936), from the winter bile of the toad is given the name **trihydroxyisosterocholenic acid** (m.p. 227°) and regarded as an isomer of trihydroxybusterocholenic acid of the formula C₂₆H₄₆O₅. The two acids yield different saturated acids on removal of the hydroxyl groups and hydrogenation.

Acids from Rabbit Bile (p. 128). From this source Kishi, *Z. physiol. Chem.*, 238, 210 (1936), isolated lithocholic acid and two new isomers of desoxycholic acid (C₂₄H₄₀O₄). One of these substances, ***α*-lagodesoxycholic acid** (Gr. *lagōs*, hare), m.p. 156-157°, yielded a hydroxyl-free saturated acid identified as cholanic acid, and on oxidation it was converted into dehydrodesoxycholic acid (3,12-diketochoLANic acid). The new acid therefore conforms to desoxycholic acid in the location of the hydroxyl groups and in the configuration of the ring system, and it must differ from this compound only in the steric arrangement of one or both of the hydroxyl groups. As the substance resembles the other bile acids in not being precipitated by digitonin, the configuration at C₃ probably is the same as in desoxycholic acid (*epi*) while that at C₁₂ is different. It is very interesting that this subtle difference apparently renders the new acid incapable of sharing with desoxycholic acid the specific property of forming choleic acids (p. 129).

The second new substance ***β*-lagodesoxycholic acid** (m.p. 213°), yields on oxidation an acid not identical with the known 3,12-, 3,7-, or 7,12-diketo acids and the structure is still unknown.

New Acid from Ox Bile (p. 128). In continuing the systematic working of large quantities of ox bile, Wieland and Hanke, *Z. physiol. Chem.*, 241, 93 (1936), isolated a substance of exceedingly weak acidity, to which the provisional formula C₂₉H₄₆O₈ is assigned. The new compound clearly is of a type quite different from the cholanic acid derivatives and it shows a marked similarity to the acidic triterpenoid saponinins (pp. 318-321). For this reason it is named **sapochoLic acid**. A

particularly close resemblance was noted to pyroquinovic acid ($C_{29}H_{46}O_8$), a decarboxylation product of the dibasic quinovic acid (p. 424). Like the sapogenins, sapocholic acid forms a water-insoluble sodium salt, it yields a bromo lactone (γ , δ -unsaturated acid group), and the ester, prepared with the use of diazomethane, is very resistant to hydrolysis.

Bile of the Alligator Turtle (p. 128). Yamasaki and Yuuki, *Z. physiol. Chem.*, **244**, 173 (1936), isolated from this source a trihydroxysterchoholic acid lactone (C_{27} or C_{28}) and a tetrahydroxy lactone (C_{28} or C_{29}).

"Choleic Acid Principle" (p. 131). A further indication that molecular compound formation plays no part in the intestinal digestion of fats or in the transportation of cholesterol in the bile is furnished by the work of Cortese and Bauman, *J. Biol. Chem.*, **113**, 779 (1936). These investigators prepared pure glycodesoxycholic acid by a process similar to that employed for the synthetic conjugation of cholic acid and glycine (p. 125) and found that the conjugated acid, unlike desoxycholic acid itself, does not form true coördinative complexes with fatty acids. Substances described in the literature as more or less stable "glycocholic acids" are regarded by Cortese and Bauman as impure specimens of the conjugated acid. In the course of the work it was noted that desoxycholic acid loses its power to form choleic acids when the hydroxyl groups are formylated or when the acid is esterified.

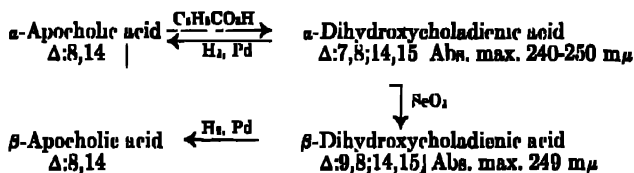
Choleic Acids (p. 132). Marx and Sobotka, *J. Org. Chem.*, **1**, 275 (1936), prepared the tetra-choleic acid of anthracene in alcohol-benzene solution. The *cis-trans* isomers oleic acid and elaidic acid have the same coördination number (8) as stearic acid. Colored choleic acids were prepared from certain colored diketones.

Apocholic Acid and Dihydroxycholenic Acid (p. 132). From the results of a study of the action of selenium dioxide in aqueous alcohol on apocholic acid and in analogy with the behavior of the isomeric ergostenols (p. 371), Callow, *J. Chem. Soc.*, 462 (1936), concluded that the formulas ascribed to the unsaturated acids derived from cholic acid require revision.

Callow considers that in 3,12-dihydroxycholenic acid the double bond probably is located at $C_{14}-C_{15}$ in ring D. This affords a satisfactory interpretation of the oxidative degradation observed by Wieland and Dane,²⁰ and it accords better with the observation that the acid is susceptible to hydrogenation. In analogy with β -ergostenol, it would be expected that an acid having the $\Delta^{7,8}$ -constitution originally attributed to dihydroxycholenic acid would undergo isomerization (to apocholic acid) and not hydrogenation. Apocholic acid, according to Callow, is a $\Delta^{8,14}$ -compound similar to α -ergostenol, which likewise is resistant to hydrogenation. The interconversion of apocholic acid and dihydroxy-

cholic acid (probably an equilibrium) is assumed to involve the migration of the double bond between adjacent positions in rings C and D, and the inert character of this linkage in the case of apocholic acid is attributed to its situation between quaternary carbon atoms (8,14). Wieland, Dietz and Ottawa, *Z. physiol. Chem.*, **244**, 194 (1936), share this view of the structures.

The formula suggested for apocholic acid is supported by the results of a study of the further dehydrogenation of this substance with perbenzoic acid and with selenium dioxide. Isomeric dihydroxycholadienic acids were obtained. The relationships between these substances and their properties, together with the structures which Callow has ascribed to them, are as follows:



The type of absorption spectrum exhibited by the doubly unsaturated acids is characteristic of compounds having two conjugated double bonds distributed between two rings (compare the isomers of ergosterol, p. 374). It is of interest that a substance obtained by the action of alcoholic potassium hydroxide on 5,6-dibromocholanic acid and assigned the structure of $\Delta^{4,8}$ -choladienic acid conforms to the same rule and exhibits an absorption maximum at about 235 m μ [Wieland and co-workers, *Z. physiol. Chem.*, **241**, 47 (1936)]. β -Apocholic acid appears to be a stereoisomer of ordinary (α -) apocholic acid and to differ from this substance only in the configuration at the asymmetric center C₉. Like the α -acid, β -apocholic acid forms stable molecular compounds with acetic acid and with xylene.

Partial Synthesis of Sterol Derivatives from Bile Acids (p. 136). Employing the method of Wieland and Jacobi,⁹⁴ who succeeded in preparing coprostanone from cholic acid by a series of reactions beginning with the condensation of the ester of the bile acid with isopropylmagnesium bromide, Reinidel and Niederländer, *Ann.*, **522**, 218 (1936), attempted to prepare a C₂₇-sterol (*epicoprostanol*) from lithocholic acid. They were unable, however, to effect the reduction of an intermediate ketone, 3-*epi*hydroxycoprostanone-24. Sterols with shorter side chains were obtained without difficulty. Using a variation of the general Grignard synthesis, Fernholz, *Ber.*, **69**, 1792 (1936), prepared from cholic acid a substance having the carbon skeleton of pseudoergostane and

apparently consisting in large part at least of the parent hydrocarbon from ergosterol.

Lithocholic Acid from Cholesterol (addition to p. 157). The preparation of a natural bile acid from cholesterol was accomplished for the first time by Schoenheimer and Berliner, *J. Biol. Chem.*, **115**, 19 (1936), the actual starting material being 3-hydroxy- Δ^5 -cholenic acid, a substance obtained as a by-product in the preparation of dehydroisoandrosterone as described on page 234 (see also p. 393). This was oxidized to 3-keto- Δ^4 -cholenic acid, which on hydrogenation (as the ester) gave a mixture of lithocholic acid and β -3-hydroxyallocholanic acid (p. 170). The mixture was separated by precipitating the latter compound (as the ester) with digitonin. Lithocholic acid is an *cpi*-compound and is non-precipitable (p. 231).

Ruzicka's Hydrocarbon "C₂₁H₁₆" (p. 158). The hydrocarbon, m.p. 275°, which Ruzicka^{44,47} isolated as one product of the dehydrogenation of cholic acid at 360°, has been identified as 5-methyl-2',1'-naphtho-1,2-fluorene by Bachmann, Cook, Hewett and Iball, *J. Chem. Soc.*, 54 (1936). Cook and co-workers⁵⁰ had been led to suspect this type of structure from spectroscopic studies, and their suggested revision of the formula to C₂₂H₁₆ was supported by X-ray crystallographic evidence.⁶² In the work cited, five methylnaphthofluorenes were synthesized for comparison (Bogert-Cook method) and the 5-isomer was found to be identical with Ruzicka's hydrocarbon. The structure of the compound corresponds to that illustrated in formula II, p. 166, except that the isopropyl group is not present. The low temperature dehydrogenation involves a simple cyclization of the cholic acid side chain at the 16-position without disturbance of any of the carbon atoms other than those of the angular methyl groups.

Dehydrogenation of Hydrindenes (p. 159). Nenitzescu and Cioranescu, *Ber.*, **69**, 1040 (1936), obtained small amounts of naphthalene on passing 1-methylhydrindene and its hexahydro derivative over palladium charcoal at 310-350°, that is, at a temperature considerably below that at which Ruzicka and Peyser⁴⁸ observed a reaction. That ring-enlargement can occur under such mild conditions emphasizes the uncertainty in structural evidence based solely upon the results of dehydrogenation. The reaction affords some analogy for the conversion of sterol derivatives into chrysene.

Formation of Spirans (p. 162). E. Bergmann and Blum-Bergmann, *J. Am. Chem. Soc.*, **58**, 1678 (1936), encountered spirans and their rearrangement products in the synthesis of cyclopentenotriphenylene by the Bogert-Cook method.

The Diels Hydrocarbon (p. 165). In a further study, Hillemann, *Ber.*, 69, 2610 (1936), reported that the highly purified compound (126-127°) is not fluorescent.

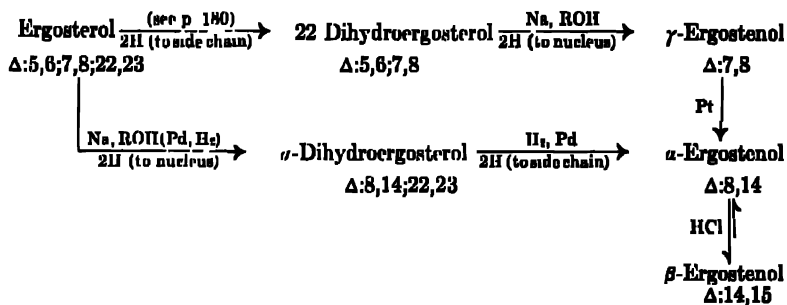
Mechanism of Dehydrogenation (p. 166). In order to test the theory that the migration of the methyl group at C₁₃ has nothing to do with the process of dehydrogenation but is due to the pyrolytic fission of the side chain at C₁₇, E. Bergmann and F. Bergmann, *Chemistry and Industry*, 55, 272 (1936) reinvestigated the thermal decomposition of cholesteryl chloride at 300° [Mauthner and Suida, *Monatsh.*, 17, 41 (1896); H. Fischer and Treibs, *Ann.*, 446, 241 (1926)]. The preliminary results support the above theory, for there was obtained a mobile distillate composed of octane and octene, corresponding to the side chain, and a main fraction from the residue having the composition of a saturated hydrocarbon, C₁₆H₃₀, corresponding to the actiocholane part of the molecule.

The Methyl Group at C₁₃ (p. 169). A rigid proof of this feature of the structure is provided by observations made in the investigation of the sex hormones, as summarized on page 387.

Ergosterol, Absorption Spectrum (p. 173). From an examination of substances having the same arrangement of conjugated double bonds as ergosterol but lacking the hydroxyl group, Dimroth and Trautmann, *Ber.*, 69, 669 (1936), found that the conjugated system of the sterol alone is responsible for the characteristic absorption bands between 260 and 290 mμ and that the hydroxyl group is without influence. The compounds examined, ergostatriene ($\Delta^{5,7,22}$) and 7-dehydrocholestene, exhibited the same type of spectrum as ergosterol, showing maxima at approximately 273 and 280 mμ. The first hydrocarbon was prepared by adding maleic anhydride to ergosterol to protect the conjugated system, oxidizing the secondary hydroxyl group of the addition product, reducing the carbonyl group by the Clemmensen method, and eliminating maleic anhydride by vacuum distillation. 7-Dehydrocholestene was obtained from 7-hydroxycholestene [Windaus, *ibid.*, 53, 495 (1920)] by the method given on page 180 for the preparation of 7-dehydrocholesterol.

Ergosterols (addition to p. 176). By various direct and indirect methods ergosterol has been converted into three well-characterized, singly unsaturated alcohols known as α -, β -, and γ -ergosterol. Since these isomers differ considerably in their stability and in their deportment under hydrogenating conditions, it is a matter of considerable interest to be able to interpret the properties in terms of specific structures. This problem has been solved in the case of β -ergosterol, and the structures suggested for the other isomers are supported by convincing, if not final, evidence. These structures are indicated in the accompanying chart,

which summarizes the methods by which the three ergosterols have been obtained.



22-Dihydroergosterol is prepared by protecting the unsaturated system by the addition of maleic anhydride and saturating the double bond in the side chain, as described on page 180, and it appears that the reduction of the substance with sodium and alcohol to γ -ergosterol involves the addition of hydrogen to the 5,6-position, leaving the 7,8-ethylenic linkage undisturbed [Windaus and Langer, *Ann.*, **508**, 105 (1934)]. In the presence of platinum or palladium catalyst, γ -ergosterol fails to absorb hydrogen but is merely isomerized to α -ergosterol, a transformation which is interpreted as involving a migration of the double bond from the 7,8- to the adjacent 8,14-position. α -Ergosterol cannot be hydrogenated under ordinary conditions and it is believed that the inert character of the double bond is due to its location between two quaternary carbon atoms, or bridge heads. A shorter route to α -ergosterol consists in the reduction of ergosterol with sodium and alcohol to α -dihydroergosterol, followed by catalytic hydrogenation of the ethylenic linkage in the side chain [Reindel and E. Walter, *Ann.*, **460**, 214 (1928)]. In the course of the reactions it appears that a nuclear double bond migrates from C₇-C₈ to C₈-C₁₄, and since α -dihydroergosterol can be obtained from ergosterol by catalytic hydrogenation [Heilbron and Sexton, *J. Chem. Soc.*, 924 (1929)] as well as with sodium and alcohol, it is probable that the migration occurs in the first step and is not caused by the catalyst employed in the addition of the second mole of hydrogen.

By the action of hydrochloric acid α -ergosterol is isomerized partially to the third compound, β -ergosterol [Reindel, E. Walter and Rauch, *Ann.*, **452**, 34 (1927); Reindel and E. Walter, *loc. cit.*; Heilbron and Wilkinson, *J. Chem. Soc.*, 1708 (1932)]. It is supposed that the isomerization consists in the migration of the double bond from C₈-C₁₄ to the adjacent position C₁₄-C₁₅ in ring D, probably by the addition and elimination of hydrogen chloride. The β -compound is the only one of the isomers which is easily hydrogenated. That the double bond of β -ergosterol occupies

the position indicated has been established with certainty. Achtermann, *Z. physiol. Chem.*, **225**, 141 (1934), ozonized the acetate (cleavage at C₁₄-C₁₅) and on pyrolysis of the product obtained a keto alcohol of the formula C₁₆H₂₆O₂. Laucht, *ibid.*, **237**, 236 (1935), fully characterized this substance by converting it by dehydrogenation with selenium into 2-methylphenanthrene, and he also isolated (as the semicarbazone) the other fragment of the pyrolysis, an α,β -unsaturated aldehyde, C₁₂H₂₂O. These products can arise only from an initial ozonolysis between positions 14 and 15.

The structures of α - and γ -ergosterol are not known with equal certainty. From the ready isomerization from the γ - to the α - to the β -form it is inferred that at each step the double bond migrates to an adjacent position, progressing from ring B to ring C, and finally to ring D.

It is of interest that on the introduction of a second nuclear double bond by treatment with perbenzoic acid, α -ergosterol [Windaus and Lüttringhaus, *Ann.*, **481**, 119 (1930)] and β -ergosterol [Morrison and Simpson, *J. Chem. Soc.*, 1710 (1932)] both yield the same compound, dehydroergosterol. The identical substance results from the isomerization of 22-dihydroergosterol with gaseous hydrogen chloride [Windaus and Langer, *loc. cit.*] Dehydroergosterol was obtained by Callow, *J. Chem. Soc.*, 462 (1936), by the action of selenium dioxide on α -ergosterol. Considering the analogy with other dehydrogenation products, Callow suggested that the double bonds occupy the conjugated positions C₈-C₉ and C₁₄-C₁₅. It is interesting that in aqueous alcoholic solution the action of selenium dioxide on sterols having one nuclear ethylenic linkage results in the introduction of an additional double bond [see also Callow and Rosenheim, *ibid.*, 387 (1933)], the reaction apparently involving the 1,4-elimination of two atoms of hydrogen. In the case of ergosterol, dehydrogenation with selenium dioxide in aqueous alcoholic solution results in the extension of the conjugated system already present, the chief product being dehydroergosterol (p. 175).

Isomers of Ergosterol (addition to p. 176). In the course of the work cited above, Callow and Rosenheim noted that α -dihydroergosterol is converted by selenium dioxide into ergosterol-D, an isomer of ergosterol which had been obtained previously from the same starting material using mercuric acetate [Heilbron, Johnstone and Spring, *J. Chem. Soc.*, 2248 (1929)], and by the reduction of a ketone prepared by heating ergosterol with nickel catalyst at 220° [Windaus and Aubagen, *Ann.*, 472, 185 (1929)]. Callow (*loc. cit.*) suggested for ergosterol-D a formula similar to that assigned to dehydroergosterol, namely, with the nuclear double bonds at the 8,9- and 14,15-positions (and with a third ethylenic linkage in the side chain). This suggestion is based partly upon the fact

that ergosterol-D has an absorption maximum at 242 $m\mu$, which is regarded as characteristic of compounds having two conjugated double bonds distributed between two rings. Ergosterol-B₂, another well-characterized isomer which can be obtained (along with the B- and B₂-compounds) by the isomerization of ergosterol with hydrogen chloride [Windaus, Dithmar, Murke and Suckfüll, *Ann.*, **488**, 91 (1931)], is regarded by both Laucht and Callow (*loc. cit.*) as having the bond structure Δ : 7,8; 14,15; 22,23. The sterol adds maleic anhydride. The suggested structure, indicating the presence of conjugated nuclear double bonds distributed between rings B and D, is consistent with the absorption spectrum (maximum at 242 $m\mu$), which is similar to that of the isomer. The presence of two conjugated ethylenic linkages in a single ring has an enhancing effect and the selective absorption occurs at a longer wave length. Ergosterol, 22-dihydroergosterol, and 7-dehydrocholesterol exhibit absorption maxima at about 280 $m\mu$. The marked difference in the absorption spectra of compounds containing the two types of diene systems should be of great value in establishing the structures of other unsaturated compounds.

Ergosterol-D and ergosterol-B, are the most fully characterized of a large number of isomers of ergosterol which have been obtained by chemical means. The study of the chemical isomerization was undertaken at a time when it seemed possible to produce in this way substances of the type formed in the process of irradiation (p. 177 ff.), and a number of methods were developed for effecting isomerizations (see Windaus, Dithmar, Murke and Suckfüll, *loc. cit.*). With the recognition that irradiation results in the opening of one of the original rings, this line of attack was abandoned.

7-Dehydrocholesterol (p. 180). In an extensive study of the reactions of 7-dehydrocholesterol, Fr. Schenck, Buchholz and Wiese, *Ber.*, **69**, 2696 (1936), found the behavior of the substance practically identical with that of ergosterol. 7-Dehydrocholesterol was converted through a "pinacol" to a substance (norsterol) analogous to neoergosterol; it was transformed into a peroxide, a maleic anhydride addition product, isomeric cholesterols, and into a series of isomers, using the standard methods developed for ergosterol. Fr. Schenck, *Z. physiol. Chem.*, **243**, 119 (1936), investigating the preparative method, isolated a by-product in the conversion of cholesteryl acetate into 7-ketocholesteryl acetate (I) and identified the substance as 3,5-diacetoxycholestanone-6.

Quantitative Microhydrogenation (p. 183). Improved forms of apparatus are described by Jackson and R. N. Jones, *J. Chem. Soc.*, 895 (1936), Zechmeister and v. Cholnoky, *Chem. Ztg.*, **60**, 655 (1936), and Mayeda, *J. Pharm. Soc. Japan*, **56**, 511 (1936).

Dihydrovitamin D₂ (p. 184). The dihydro derivative resulting from the reduction of vitamin D₂ with sodium and propyl alcohol has been found by v. Reichel and Deppe, *Z. physiol. Chem.*, **239**, 143 (1936), to yield a crystalline trioxide on reaction with perbenzoic acid. A study of the further oxidation of the substance with chromic anhydride furnished evidence that one oxidic linkage is located at C₂₂-C₂₃ and another at C₃-C₁₀. This accounts for all but one of the double bonds of dihydrovitamin D₂, and since the substance does not add maleic anhydride and exhibits no selective absorption in the ultraviolet region the third ethylenic linkage evidently does not occupy a position of conjugation and probably is at C₇-C₈, corresponding to one of the original centers of unsaturation in vitamin D₂ (formula VI, p. 185). It appears, therefore, that the reduction with sodium and alcohol consists in the addition of hydrogen at C₈ and C₁₄, that is, at the ends of a 1,4-conjugated system.

Lumisterol (p. 184). Although the evidence is not entirely complete, there are strong indications that lumisterol differs from ergosterol not in the positions of the double bonds or in the spatial arrangement of the hydroxyl group at C₃, but in the configuration at the asymmetric center C₁₀ carrying the angular methyl group. That the nuclear ethylenic linkages are conjugated is shown perhaps most clearly by the observation of Heilbron, Spring and Stewart³¹ that an additional double bond can be introduced by the action of mercuric acetate. It has been shown, moreover, by Dimroth, *Ber.*, **69**, 1123 (1936), that the resulting dehydrolumisterol has the same characteristic absorption spectrum, indicative of three conjugated double bonds, as dehydroergosterol (p. 175). If lumisterol contains a conjugated system, the character of the triol obtained by Heilbron, Spring and Stewart, and of a stereoisomeric triol studied by Dimroth, locates this system as extending from C₃ to C₈, exactly as in the case of ergosterol. The fact that lumisterol fails to form an insoluble digitonide would suggest (as stated on page 186) that this photoisomeride differs from ergosterol only in an epimeric arrangement of the hydroxyl group. If this were the case both sterols should yield the same unsaturated hydrocarbon on dehydration, but Heilbron, Spring and Stewart found that they yield different products.

For these reasons, Dimroth considers that lumisterol must have the same structure and the same configuration at C₃ as ergosterol. The change produced on irradiation must consist in a steric inversion at some center other than C₃, and most probably at a position coming under the activating influence of the light-absorbing conjugated system. Carbon atoms 9 and 10 are the most likely centers, since they are adjacent to double bonds, and since they are known to be involved in the ultimate rupture of the tetracyclic system. A further inference can be drawn

from the consideration that on the basis of the evidence available dehydrolumisterol very probably has the same structure as dehydroergosterol (see formula, p. 175). Since in these compounds there is no longer a center of asymmetry at C_6 , they must differ only in the configuration at C_{10} . It is inferred that lumisterol differs from ergosterol in this same respect.

Further observations of Dimroth lend weight to this view. Dehydroergosterol, he found, yields a perhydro derivative different from lumistanol, the hydrogenation product of lumisterol. Since carbon atom 10 retains its asymmetry throughout the changes involved, while the center C_6 becomes unsaturated in the former process and hence available for steric inversion in the hydrogenation, lumistanol and perhydrodehydrolumisterol can differ only in the configuration at C_6 . Since neither substance is identical with ergostanol, they must differ from this compound in the configuration at the alternate center C_{10} , in conformity with the conclusion reached above.

Dimroth also made the striking discovery that perhydrodehydrolumisterol is identical with the perhydro derivative of pyrocalciferol. It is significant that the re-formation of the tetracyclic system involves the closing of a ring between the carbon atoms (9 and 10) assumed above to be involved in the first stage of the process of photoisomerism. Ring closure could result in configurations at C_6 and C_{10} different from the original ones. It appears from Dimroth's observation that the arrangement of the methyl group of pyrocalciferol is the same as in lumisterol, and it also is evident that no steric inversions occur in other parts of the molecule in the transformation of lumisterol through tachysterol to Vitamin D_2 .

It is now evident that the assumption of an epimeric arrangement of the hydroxyl group of lumisterol and of the other isomerides of the series, based on the failure of these substances to give insoluble digitonides, is incorrect. Dimroth considers that the compounds all have the normal arrangement at C_3 of ergosterol and that the inability to combine with digitonin may be due to the abnormal spatial arrangement of the methyl group at C_{10} .

Vitamin D_2 or Calciferol (p. 185). Heilbron, R. N. Jones, Samant and Spring, *J. Chem. Soc.*, 905 (1936), confirmed the structure VIII for the aldehydic oxidation product by further analyses and by the observation that the absorption spectrum of the semicarbazone is that of an α , β -unsaturated compound. They further investigated the ozonolysis of vitamin D_2 (calciferol) and isolated as one product a keto acid ($C_{13}H_{20}O_3$) which must result from the fission of the molecule at the C_7-C_8 and the $C_{22}-C_{23}$ ethylenic linkages. This can only be represented

by a formula similar to X but having the side chain $-\text{CH}(\text{CH}_3)\text{CO}_2\text{H}$. As a second product of ozonolysis, formaldehyde was isolated and characterized as the dimedon derivative (the condensation product with two molecules of dimethyldihydroresorcinol). This establishes the presence of an exocyclic methylene group, as postulated by Windaus and Thiele³⁹ (formula VI), and removes any uncertainty in the evidence adduced by these investigators arising from the possibility of a rearrangement in the course of the Diels-Alder condensation or during the drastic pyrogenic reaction. The observations of the English investigators were further confirmed and extended by Windaus and W. Grundmann, *Ann.*, **524**, 295 (1936), who isolated as oxidation products of vitamin D₂ the aldehyde $\text{C}_{21}\text{H}_{34}\text{O}$, the keto acid $\text{C}_{14}\text{H}_{20}\text{O}_4$, and an unsaturated ketone ($\text{C}_{10}\text{H}_{16}\text{O}$) which on hydrogenation yielded the ketone X (p. 185) of Windaus and Thiele. Windaus and Grundmann also obtained 20% of the theoretical amount of formic acid in the permanganate oxidation of vitamin D₂, and about 30% of the calculated amount of formaldehyde was obtained using ozone. Since ergosterol under similar conditions gives a few per cent of formic acid and a trace of formaldehyde, even though it contains no methylene group, Windaus regards the isolation of the other oxidation products as providing the most secure indication of the structure.

With the body of evidence now available the structure of the active irradiation product of ergosterol is clearly established in all details. In the light of Dimroth's work on lumisterol (p. 375), the formula for vitamin D₂ reproduced on pages 184 and 185 should be revised to indicate that the substance has the normal configuration at C₁ and is not an *epi*-compound.

Vitamin D (p. 186) Since the publication of the first edition the nature of the antirachitic principle of fish liver oils finally has been established beyond question as the result of further chemical and biological investigations of the problem. At the time of the previous review vitamin D₂ (calciferol), the active product of the irradiation of ergosterol, had been well characterized as to structure, and it was recognized that the substance probably is not identical with natural vitamin D but is a good substitute for it. Active substances differing from vitamin D₂ only in relatively minor features of structure had been obtained also by the irradiation of 22-dihydroergosterol and of 7-dehydrocholesterol. Since these substances, of at least qualitatively similar biological actions, resemble one another closely in their structures and absorption spectra, it seemed likely that the antirachitic agent found in natural sources also is of the same general type. On the other hand, the optical properties noted for purified vitamin D concentrates seemed to indicate a striking dis-

parity between the natural vitamin and the antirachitically active substances prepared from sterols (p. 179).

Attempts to identify natural vitamin D by comparing the biological actions of fish liver oils with those of known irradiation products were undertaken at an early date and, thanks to the efficacy of a special method of comparison, considerable progress was made. The method depends upon the fact that two substances of qualitatively similar physiological actions may differ appreciably in their relative potency when administered to different types of animals. Massengale and Nussmeier, *J. Biol. Chem.*, **87**, 423 (1930), were the first to compare vitamin D from cod liver oil with irradiated ergosterol by this method, and they found that, on the same rat-unit basis, irradiated ergosterol has less antirachitic effect than cod liver oil when tested on chicks. The conclusion that vitamin D and D₂ are not identical was confirmed by Steenbock, Kletzien and Halpin, *ibid.*, **97**, 249 (1932), and by others. Similarly, Waddell, *ibid.*, **105**, 711 (1934), observed that irradiated crude cholesterol is more effective in preventing rickets in chicks than an equivalent number of rat units of irradiated ergosterol [see also Dole, *Z. Vitaminforsch.*, **5**, 161 (1936)]. F. C. Koch, E. M. Koch and Ragins, *J. Biol. Chem.*, **85**, 141 (1929), found that cholesterol which has been purified through the dibromide shows increased activatability after it has been heated at 200° with traces of oxygen, and on comparing the rat and chick units Hathaway and Lobb, *ibid.*, **113**, 105 (1936), and Haman and Steenbock, *ibid.*, **114**, 505 (1936), found that irradiated heat-treated cholesterol contains a vitamin resembling that of liver oils more closely than irradiated ergosterol does. [Studies of the chemical activation of cholesterol are reviewed by Yoder, *ibid.*, **116**, 71 (1936). This line of work was initiated by the observation of Bills, *ibid.*, **67**, 753 (1926), that a weak antirachitic activation can be accomplished by treatment of cholesterol with the fuller's earth, floridin.]

The biological studies had reached a point where it appeared profitable to reinvestigate the provitamin of crude cholesterol, to attempt the isolation of the provitamin of heat-treated cholesterol, and to compare vitamin D concentrates with the irradiation product of 7-dehydrocholesterol by differential bio-assay, but at this phase in the work the problem was solved in investigations of a chemical nature conducted at the Göttingen laboratory.

The difficult task of isolating the antirachitic principle of tunny liver oil in a pure form was accomplished by Brockmann, *Z. physiol. Chem.*, **241**, 104 (1936). It seemed reasonable to suppose that the natural vitamin would bear a fairly close resemblance to vitamin D₂ in structure, even though it might not be identical with this substance; and Brock-

mann, assuming that there would be a fair correspondence in the solubilities, adsorbabilities, absorption spectra, and other properties of the two substances, designed a procedure for the isolation of vitamin D based upon a number of trial experiments with vitamin D₂. The preliminary work included the development of a colorimetric method for the quantitative determination of vitamin D₂ (or D) based upon the measurement of the intensity of a characteristic absorption band at 500 m μ of an antimony trichloride complex. [Brockmann and Y. H. Chen, *ibid.*, 241, 129 (1936). For comments concerning the use and history of this colorimetric method, see F. A. Robinson and F. E. Young, *Chemistry and Industry*, 55, 835 (1936). For a colorimetric determination employing aluminum chloride, see Tzoni, *Biochem. Z.*, 287, 429 (1936)]. Since the complex from vitamin A has an absorption maximum at 620 m μ the presence of this substance does not interfere with the determination. Tachysterol is the only related substance giving the specific test.

A liver oil concentrate containing by assay 0.32% of vitamin D was used as the starting material, and on distribution between 90% methanol and ligroin the bulk of the vitamin A was retained in the former solvent while the vitamin D collected in the ligroin. Extraction of the ligroin solution with 95% methanol removed the vitamin D, and much inactive material was retained in the ligroin. A further enrichment was accomplished by chromatographic adsorption on aluminum hydroxide, a dye of the same degree of adsorbability as vitamin D₂ being used as an indicator to reveal the adsorption zone. Finally the dye was removed with alkali, cholesterol was precipitated as the digitonide, and the active principle was esterified with 3,5-dinitrobenzoyl chloride. After purification by adsorption, a crystalline, 3,5-dinitrobenzoate, m.p. 128-129°, was isolated. Hydrolysis gave a non-crystalline sterol having an ultraviolet absorption spectrum similar to that of vitamin D₂ (maximum at 265 m μ and an antirachitic activity of the same order of magnitude. The crystalline ester was not identical with that of the active irradiation product of ergosterol and, indeed, it differed from this substance in composition. The composition, however, was that of the 3,5-dinitrobenzoate of an antirachitic substance known as vitamin D₃, which Windaus, Fr. Schonck and v. Werder, *Z. physiol. Chem.*, 241, 100 (1936), had just prepared by the irradiation of 7-dehydrocholesterol (p. 180). The two esters melted at the same temperature and direct comparison established their complete identity. Grab, *ibid.*, 243, 63 (1936), later compared the antirachitic activity of Brockmann's product with that of vitamin D₃, using both rats and chicks, and found that the effective dosages agreed so closely that the identity of the biological activities cannot be questioned. He also established a quantitative correspondence in the biological actions

of tunny liver oil and crystalline vitamin D₃ and thus provided independent evidence of the identity of the natural vitamin.

The isolation of the natural vitamin and its prompt identification by comparison with a substance which already had been produced by chemical means, has finally solved a most important and perplexing problem. The natural substance, or vitamin D₃ from 7-dehydrocholesterol, can be assigned with assurance a structure similar to that of vitamin D₂ (formula VI, p. 185) but having the saturated side chain of cholesterol ($-C_8H_{17}$). The mother substance of the vitamin very probably is cholesterol, but the manner in which the transformation occurs is not yet clear. It seems hardly possible that the vitamin arises from the irradiation of a provitamin in the fish, but only physiological investigation can determine whether the final vitamin is acquired from irradiated food, or by a synthesis requiring no radiant energy in the organism of the fish. From a comparison of the absorption spectra, E. M. Koch and F. C. Koch, *J. Biol. Chem.*, **116**, 757 (1936), concluded that the activatable contaminant of crude spinal cord cholesterol probably is 7-dehydrocholesterol, but that the provitamin D of heated, purified cholesterol is a distinctly different substance.

From the work of Brockmann it is clear that the early reports of the properties of natural vitamin D (Ender, Rygh, pp. 178, 179) are erroneous. The substance is optically active, it has an absorption spectrum characteristic of sterols with three conjugated double bonds, and it readily adds maleic anhydride. According to a preliminary report of Haslewood and Drummond, *Chemistry and Industry*, **55**, 598 (1936), still other substances of antirachitic activity may be present in fish liver oils. Brockmann's observations, however, have been confirmed by Simons and Zucker, *J. Am. Chem. Soc.*, **58**, 2655 (1936), who isolated from tunny liver oil, by a process not involving chromatographic technique, a substance whose 3,5-dinitrobenzoate melted at 128.5° and which differed only slightly from Brockmann's compound in biological activity and in the character of the absorption band. Neraacher and Reichstein, *Helv. Chim. Acta*, **19**, 1382 (1936), obtained from the same source three crystalline dinitrobenzoates, but the alcohols proved to be inactive. Searching for the provitamin present in about 0.18% in crude cholesterol from egg yolk, Windaus and Stange, *Z. physiol. Chem.*, **244**, 218 (1936), isolated ergosterol. They consider it probable that this sterol is resorbed in small amounts from the diet and does not arise in the organism of the hen.

Relationship Between Structure and Antirachitic Activity. It is now known that irradiated 22-dihydroergosterol (contrary to the preliminary reports, p. 180), is more potent than vitamin D₂ and nearly as active as vitamin D₃ [Grab, *loc. cit.*; McDonald, *J. Biol. Chem.*, **114**, Proc.

Lxv (1936)]. The extra methyl group in the side chain appears to have but little influence in determining the physiological potency. According to Grab's results, vitamin D₂ is somewhat less potent than vitamin D₃, indicating that the double bond of the side chain detracts from the activity. The most essential feature of structure doubtless is the presence of a system of three conjugated double bonds extending between the original rings A and C, and in order for a sterol to function as a provitamin it evidently must contain a diene system between positions 5 and 8. In extension of these observations, additional sterols of the type defined have been investigated at the Göttingen laboratory. 7-Dehydrostosterol [Wunderlich, *Z. physiol. Chem.*, **241**, 116 (1936)] gave on irradiation a substance of high antirachitic activity, but, surprisingly enough, the irradiation product obtained from 7-dehydrostigmasterol [Linsert, *ibid.*, **241**, 125 (1936)] proved to be inactive, or at the most very feebly active. Bann, Heilbron and Spring, *J. Chem. Soc.*, 1274 (1936), attempted to prepare a similarly unsaturated sterol by a different method but encountered an unexpected obstacle. Treatment of 7-ketocholesteryl acetate (formula I, p. 180) with three equivalents of methylmagnesium iodide gave 7-hydroxy-7-methylecholesterol, but on dehydration of this carbinol (as the benzoate) an ethylenic linkage was introduced not at the desired 7,8-position, but in the alternate exocyclic position. Irradiated 7-methylenecholesterol, as anticipated, is antirachitically inactive. In another investigation Barr, Heilbron, Parry and Spring, *ibid.*, 1437 (1936) discovered a new route to 7-dehydrocholesterol. Noting that cholesterol is remarkably stable to neutral potassium permanganate, these workers tried oxidizing cholesteryl hydrogen phthalate with alkaline permanganate. Of three oxidation products isolated, one proved to be the acid phthalate of a 7-hydroxycholesterol, m.p. 184-185°, for the diol formed a dibenzoate which yielded 7-dehydrocholesteryl benzoate on pyrolysis. The new diol is a stereoisomer of the 7-hydroxycholesterol of Windaus, Lettré and Schenck,²⁴ the difference being in the configuration at C₇. To distinguish the isomers, the diol of Windaus, Lettré and Schenck is appropriately named 7(α)-hydroxycholesterol, while that of the English investigators is the 7(β)-hydroxy derivative.

Other Studies (addition to p. 186). Vangelovici and Vasiliu, *Bul. Soc. Chim. România*, **17**, 249 (1935), prepared 6-aminocholestanol and investigated other nitrogen-containing sterol derivatives. It was observed that ergosterol is converted by the action of phosphorus trichloride into an ergostatetraene (C₂₈H₄₂), m.p. 102°. This forms a hydrogen chloride addition product, m.p. 107°. Further studies of nitrogen-containing bile acid derivatives are reported by M. Schenck, *Z. physiol. Chem.*, **239**, 135 (1936); **242**, 81, **244**, 245 (1936). Lettré and Hagedorn, *ibid.*, **242**,

210 (1936), investigated synthetic glycosides of sterols in the hope of defining the features of structure responsible for the hemolytic action of the neutral saponins of the digitalis group (p. 322), but substances of a high degree of water-solubility have not yet been obtained. Dane and Brady, *ibid.*, 244, 241 (1936), prepared the 3-glucoside of desoxycholic acid with the view of increasing of the digitalis-like action of this bile acid.

Chapter V

Combined Form of Oestriol (p. 194). S. L. Cohen and Marrian, *Biochem. J.*, 30, 57 (1936), isolated from human pregnancy urine a water-soluble, ether-insoluble substance having the properties and approximately the composition of an oestriol glucuronic acid ($C_{24}H_{42}O_9$). S. L. Cohen, Marrian and Odell, *ibid.*, 30, 2250 (1936), later isolated the pure crystalline sodium salt of oestriolglucuronide and showed that the glucuronic acid is joined to the oestriol by a glucosidic link to one of the two secondary hydroxyl groups, the phenolic hydroxyl being free. The oestriol in the glucuronide has only about 1/17 the potency of the same amount of uncombined oestriol.

Colorimetric Determinations (pp. 194-195). Schmulovitz and Wylie, *J. Lab. Clin. Med.*, 21, 210 (1935); *J. Biol. Chem.*, 116, 415 (1936), used the orange or red colors of the azo dyes, formed by coupling oestrone and oestriol with diazotized *p*-nitroaniline or sulfanilic acid, as a means of estimating these substances in extracts of human pregnancy urine. Chevallier, Cornil and Verdollin, *Bull. acad. méd.*, 114, 171 (1935), used the characteristic ultraviolet absorption band at 280 $m\mu$ to detect oestrogenic substances in urinary extracts. Pincus, Wheeler, G. Young and Zahl, *J. Biol. Chem.*, 116, 253 (1936), compared several of the previous colorimetric methods of estimation and developed a new test based upon a color reaction with benzoyl chloride. A more intense color is obtained with oestrone than with oestriol, and the test distinguishes these substances from oestradiol (p. 213), which does not give the typical color.

Derivatives of Oestrone (p. 195). Dirscherl, *Z. physiol. Chem.*, 239, 49 (1936), prepared the ethyl carbonic ester of oestrone ($ROCO_2C_2H_5$), m.p. 115°, corr., by the action of ethyl chloroformate and pyridine on the hormone or by interaction with phosgene and pyridine, followed by alcoholysis of the chloroformyl ester ($ROCOCl$). The chloroformyl ester, m.p. 101-102°, was found to have an enhanced oestrogenic activity.

δ -Follicular Hormone (p. 195). The substance described by Schwenk and Hildebrandt²⁰ was later separated from the urine of mares by Win-

tersteiner, Schwenk and co-workers and found to be a mixture from which one component was isolated in a pure form through the pierate [Wintersteiner, Schwenk and Whitman, *Proc. Soc. Exptl. Biol. Med.*, **32**, 1087 (1935); Wintersteiner, Schwenk, Hirschmann and Whitman, *J. Am. Chem. Soc.*, **58**, 2652 (1936)]. The new compound, m.p. 215-217°, has the composition $C_{18}H_{20}O_2$ and was characterized as a diol having the structure of a dihydro derivative of equilenin (p. 202). The phenolic monobenzoate on oxidation gave a ketone identical with equilenin benzoate. The dihydroequilenin from urine possesses only about one-half the oestrogenic potency of equilenin (the potent, oily diol of David, p. 215, may have contained stereoisomers).

Standardization of the Sex Hormones (p. 196). At the second conference held under the auspices of the League of Nations, a second international unit, the "benzoate unit," was defined as the specific activity of 0.1 γ of a standard preparation of the pure monobenzoate of oestradiol, (p. 214) m.p. 194-195° [*Z. angew. Chem.*, **48**, 805 (1935)]. The international unit for the male hormones is defined in terms of the activity of 0.1 mg. of a pure androsterone preparation measured in the comb growth test. For the corpus luteum hormones the unit of activity is that of 1 mg. of β -progesterone (Corner-Allen or Clauberg test).

Extraction of Oestrone (p. 197). A number of improvements in the process of Beall and Marrian³⁴ are described by Beall and Edson, *Biochem. J.*, **30**, 577 (1936).

Reagents for the Isolation of Ketones (p. 197). A convenient procedure for the preparation of the Girard reagent in 90% yield starting with ethylchloroacetate, trimethylamine, and hydrazine hydrate is described by Girard and Sandulesco, *Helv. Chim. Acta*, **19**, 1095 (1936). The reagent, trimethylaminoaceto-hydrazide hydrochloride (betaine-hydrazide hydrochloride) must be stored with some care as it is hygroscopic and undergoes decomposition in a moist atmosphere. The condensation is best carried out in alcoholic solution containing 10% acetic acid, refluxing for one-half hour usually being sufficient. The condensation products from ketones are hydrolyzed very easily by mineral acids, while those from aldehydes are so stable to acid hydrolysis that a separation of ketones from aldehydes can be made without difficulty. No practical process was found for the regeneration of aldehydes. Diaryl ketones react only very slowly with the reagent. Girard and Sandulesco developed a new test for ketones based upon the formation of a colored precipitate on adding mercuric and potassium iodides to an aqueous solution of the condensation product; and it is reported that the presence of a few γ of oestrone can be recognized easily by this method in a micro test. These investigators describe a second reagent which differs from the

first only in the replacement of the trimethylamine residue by pyridine, and as it is not hygroscopic the substance is recommended for technical isolations. It is not suitable for use in the qualitative precipitation test.

Anchel and Schoenheimer, *J. Biol. Chem.*, **114**, 539 (1936), found the Girard reagent somewhat unsatisfactory for the isolation of cholestanone because the condensation product is hydrolyzed with such great ease as to make separations difficult. [See, however, Reichstein, *Helv. Chim. Acta*, **19**, 1107 (1936)]. They investigated reagents whose condensation products form water-soluble alkali salts, and found two compounds which offer certain distinct advantages, particularly when used in combination with one another. One of these, carboxymethoxylamine ($\text{H}_2\text{NOCH}_2\text{CO}_2\text{H}$), is easily prepared from the sodium derivative of acetoxime and ethyl chloroacetate [Borek and Clarke, *J. Am. Chem. Soc.*, **58**, 2020 (1936)]. It reacts rapidly with sterol ketones in alcoholic solution, and the products of the type $\text{R}_2\text{C}=\text{NOCH}_2\text{CO}_2\text{H}$ can be extracted from mixtures by distribution between potassium carbonate solution and ether, the product separating as a precipitate when the aqueous layer is acidified. The oxime acids are not attacked by air, and this is a convenient form in which to isolate the total ketones from unsaponifiable material. After regeneration of the total ketones, a separation of the α , β -unsaturated ketone cholestenone from cholestanone and coprostanone can be effected with the use of a second reagent, *p*-carboxyphenylhydrazine ($\text{H}_2\text{NNHC}_6\text{H}_4(\text{CO}_2\text{H})$). While the hydrazones of the saturated ketones are easily cleaved on refluxing the mixture with alcoholic formaldehyde solution, the hydrazone of cholestenone is not attacked under these conditions. This ketone can be recovered by refluxing in an alcoholic solution of pyruvic acid. *p*-Carboxyphenylhydrazine is not used in the first, gross extraction because the phenylhydrazones are autoxidizable, making it necessary to work as far as possible in the absence of air.

Influence of Oestrin on Plant Growth (pp. 199-200). In further investigations the results have been almost entirely negative. [Harder and Störmer, *Nachr. Ges. Wiss., Göttingen*, **11** (1934); Störmer, *Biochem. Z.*, **285**, 29 (1936); Teodoro and Zampetti, *Arch. ist. biochim. ital.*, **7**, 423 (1935); Tineker, *Ann. Appl. Biol.*, **22**, 619 (1935); Chouard, *Compt. rend. soc. biol.*, **122**, 823 (1936)]. Positive results are reported by Scharrer and Schropp, *Biochem. Z.*, **281**, 314 (1935).

X-Ray Analysis (p. 206). X-ray crystallographic data for oestrone and other hormones are given by Bernal and Crowfoot, *Z. Krist.*, **93**, 464 (1936).

Hydrogenation of Oestrone (p. 213). On hydrogenating the hormone or its acyl derivatives in the presence of Adams' catalyst in neutral or alkaline alcoholic solution, Diracherl, *Z. physiol. Chem.*, **239**, 53

(1936), obtained oestradiol (m.p. 174-175°) as the sole product. On conducting the reaction at room temperature in acetic acid solution or in alcohol containing hydrochloric acid, he found that the aromatic nucleus is attacked, usually even before the carbonyl group, giving mixtures of ketonic hexahydro derivatives, octahydro derivatives, and hexahydrodesoxyoestrones. An oestradiol-3,17, m.p. 210-211°, was isolated in a pure condition from the mixture of isomeric octahydro compounds, and a ketonic hexahydro derivative was isolated as the semicarbazone.

Isolation of Oestradiol (p. 214). Details of the isolation of the hormone are reported by MacCorquodale, Thayer and Doisy, *J. Biol. Chem.*, **115**, 435 (1936). In the most satisfactory procedure oestradiol was isolated not as the (mono) *m*-bromobenzoate, prepared by the Schotten-Baumann reaction, but as the di- α -naphthoate, the slight solubility of which in ethyl alcohol permits a ready separation from contaminating substances. The preparation of the derivative was carried out in pyridine solution and the excess α -naphthoyl chloride was removed by adding excess glycine and subsequently extracting the resulting α -naphthoylglycine with sodium bicarbonate solution. The free hormone recovered after hydrolysis (m.p. 171-172°) was identical in melting-point characteristics and in bio-assay with a sample of oestradiol (m.p. 172-173°) prepared in quantitative yield by the hydrogenation of oestrone in the presence of Adams' catalyst. The complete recovery as oestradiol of all the oestrogenic material of the four tons of sow ovaries processed in the course of the work would have given only 25 mg. of the hormone, and the isolated material amounted to about one-half of this quantity.

Not long after the discovery of the hormone by Doisy and co-workers, Wintersteiner, Schwenk and Whitman, *Proc. Soc. Exptl. Biol. Med.*, **32**, 1087 (1935), reported isolating oestradiol from the pregnancy urine of mares.

Supposed Activity of Degradation Products (p. 215). The early report that certain oxidation products of oestriol methyl ether are more active than oestrone has been retracted. MacCorquodale, Levin and Thayer, *J. Biol. Chem.*, **105**, lv (1934), state that none of the degradation products of oestrone and oestriol investigated has any significant oestrogenic activity.

Oestrogenic Activity of Synthetic Compounds (pp. 215-217). A further investigation of 9,10-dialkyl-9,10-dihydroxy-9,10-dihydro-1,2,5,6-dibenzanthracenes (III, p. 216) and related diols has been made by Cook, Dodds and Lawson, *Proc. Roy. Soc.*, (London), **B121**, 133 (1936). Seven new dialkyl derivatives of the type III were examined for oestrogenic activity, but the potency in no case equaled that of the di-*n*-propyl diol, the most active of the substances previously described. Branching of

the side chains results in a marked decrease in the activity, the di-*i*-propyl compound being only one-tenth as active as the di-*n*-propyl diol. The introduction of double bonds produces an even more pronounced change in the biological actions of the molecule, for the di-allyl compound gave entirely negative results when injected into ovariectomized rats in 10 mg. doses. An interesting test was made of the effect of cyclization of the aliphatic chain. While the di-*n*-amyl compound is inactive in large doses, the dicyclopentyl compound gave positive results in 0.5 mg. doses. The English investigators note that the configurations of the diols have not been determined and that it is not yet known if the substances studied are *cis* or *trans* compounds, or mixtures. Differences in the stereoisomeric type or in the composition of mixtures might seriously obscure the correlation of structure with biological activity. Whether the synthetic diols are single individuals or mixtures, they reproduce all the known biological actions of naturally occurring oestrone. The substances produce vaginal changes in rats and mice, they stimulate uterine enlargement and induce premature puberty in immature animals, they produce plumage changes in capons (p. 216), and reproduce pathological changes resulting from the administration of oestrone [p. 218. Burrows, Ref. 99, *British Journal of Surgery*, 23, 658 (1935)]. Cook, Dodds and Lawson also found that the di-*n*-propyl diol, like oestrone, can prepare the uterus for the endometrial proliferation brought about by progesterone (pp. 241-242), and Wolfe, *Am. J. Physiol.*, 115, 665 (1936), observed that administration of the diol to castrated female rats prevents the appearance of castration cells in the anterior lobe of the pituitary.

Other dialkyl diols of analogous structure were prepared by the addition of Grignard reagents to anthraquinone, phenanthrenequinone, chrysenequinone, 1,2-benzanthraquinone, and the *Bz*-tetrahydro derivatives of 1,2- and 2,3-benzanthraquinone. Oestrogenic activity of a low order was shown by the diphenyl diols from chrysenequinone and 1,2-benzanthracene, while all other members of these series were inactive. In the course of the work 1-hydroxy-1,2,3,4-tetrahydrophenanthrene was found inactive in rats in doses of 100 mg.

While all of the previously described oestrogenic agents except the weakly acting 1-ketooctahydroanthracene IV (p. 217) contain the phenanthrene ring system, Dodds and Lawson, *Nature*, 137, 996 (1936), discovered that a number of substances not related to phenanthrene exhibit definite oestrogenic activity [see also Dodds, *Helv. Chim. Acta*, 19, E49 (1936)]. 7,8-Dihydroxy-7,8-di-(α -naphthyl)-acenaphthene, administered in 100 mg. doses, will maintain a rat in full oestrus for a prolonged period, and it is active in doses as small as 10 mg. A number of other

dihydroxy compounds were found to possess definite oestrogenic activity when injected in rats in 100 mg. dosage, the list including such comparatively simple compounds as 4,4'-dihydroxydiphenyl, 4,4'-dihydroxydiphenylmethane, and di-(α -naphthyl)-carbinol. The effective dosages of these compounds, as far as determined, are several hundred times larger than for the dialkyl diols of the dibenzanthracene series, but the results illustrate further the absence of a high degree of structural specificity in this type of female hormonal activity.

Pincus and Werthessen, *Science*, **84**, 45 (1936), developed a special method of assay dependent upon the intraperitoneal, rather than subcutaneous, injection of the substance tested. While the results have not been correlated with those obtained by the standard Allen-Doisy technique, the method appears to offer a preliminary guide in the study of synthetic substances. Of a number of phenanthrene and hydrophenanthrene derivatives tested, the most active was 6,7-dihydroxy-1,2,3,4,9,10,11,12-octahydrophenanthrene-11,12-dicarboxylic anhydride [Fieser and Hershberg, *J. Am. Chem. Soc.*, **58**, 2314 (1936)], which was rated as more potent than 1-keto-1,2,3,4-tetrahydrophenanthrene.

Oestrone from Ergosterol (p. 219). The conversion of dehydroneoergosterol into oestrone has been realized by Marker, O. Kamm, Oakwood and Laucius, *J. Am. Chem. Soc.*, **58**, 1503 (1936); paper XI in press. It was found that on reduction with sodium and amyl alcohol dehydroneoergosterol is attacked largely at ring B, giving a phenolic tetrahydro derivative. Applying Ruzicka's method for the elimination of the sterol side chain (p. 227), this compound was oxidized in the form of the acetate and yielded, after hydrolysis, a substance identical with natural oestrone. The yields realized in these operations are not reported. By improved procedures, ergosterol was converted through ergopinacol and neoergosterol into the required dehydroneoergosterol with an overall yield of about 4% by weight. Whether or not the method proves of practical importance for the preparation of oestrone, the observation is of considerable significance in providing a clear proof that the configuration at the asymmetric centers C_{13} and C_{14} is the same in oestrone as in ergosterol and therefore in cholesterol. The certain evidence that oestrone contains an angular methyl group at C_{13} and a carbonyl group at C_{17} (pp. 211-212) now affords a rigid proof of the location of the corresponding methyl group and of the side chain in the sterols.

Bardhan's Synthesis (p. 220). In another preliminary announcement Bardhan, *Chemistry and Industry*, **55**, 879 (1936), has reported a further variation of the scheme of synthesis illustrated in formulas I-III. Phenylethyl bromide was condensed with ethyl α -acetyl- β -ketopimalate to give ethyl α -(β -phenylethyl)- β -ketopimalate. This was cyclized with

sulfuric acid to a Δ^1 -dihydronaphthalene derivative. The third ring was closed by an ester condensation with sodium, and 1-keto-1,2,3,4,9,10-hexahydrophenanthrene was obtained on hydrolysis. Details of the preparation of 3'-keto-3,4-dihydro-1,2-cyclopentenophenanthrene (III) are reported by Bardhan, *J. Chem. Soc.*, 1848 (1936).

Derivatives of Phenanthrene-1,2-dicarboxylic Anhydride (p. 221). By application of the standard synthesis (see VIII \longrightarrow IX) to suitably substituted naphthalene derivatives, A. Cohen, Cook and Hewett, *J. Chem. Soc.*, 52 (1936), prepared 7-methoxyphenanthrene-1,2-dicarboxylic anhydride, and Fieser and Herschberg, *J. Am. Chem. Soc.*, 58, 2314, 2382 (1936) prepared the 9-methoxy and the 5,9-dimethoxy derivatives of the anhydride. Starting with the ester of phenanthrene-1,2-dicarboxylic acid, L. F. Fieser, M. Fieser and Herschberg, *ibid.*, 58, 2322 (1936), prepared 1',3'-diketo-1,2-cyclopentenophenanthrene by condensation with ethyl acetate and saponification of the diketo ester. The 5,9-dimethoxyphenanthrindanedione was prepared in a similar manner [*ibid.*, 58, 2382 (1936)]. A. Cohen, *Nature*, 136, 809 (1935), prepared phenanthrene-1,2-dicarboxylic anhydride and its 7-methoxy derivative by an interesting application of the Diels-Alder reaction. It was found that 1-vinylnaphthalene combines readily with maleic anhydride at ordinary temperature to give a tetrahydrophenanthrene-1,2-dicarboxylic anhydride. The fully aromatic compound was obtained by dehydrogenation with platinum black. A similar synthesis was achieved with 7-methoxy-1-vinylnaphthalene.

7-Methoxy-1-ketotetrahydrophenanthrene (p. 221). Two alternate methods have been described for the synthesis of the compound of Butenandt and Schramm⁹ (ether of XI). A. Cohen, Cook and Hewett, *J. Chem. Soc.*, 52 (1936), converted β -(6-methoxy-1-naphthyl)-ethyl alcohol (p. 210) into γ -(6-methoxy-1-naphthyl)-butyric acid by the malonic ester synthesis and cyclized the product with 80% sulfuric acid. Hieberland, *Ber.*, 69, 1380 (1936), prepared the same intermediate in still another manner. 6-Methoxy-1-tetralone was converted by the Reformatsky condensation with ethyl bromoacetate, followed by dehydration and reduction with sodium and alcohol, into β -(6-methoxy-1-tetralyl)-ethyl alcohol. The chain was lengthened by the malonic ester synthesis and γ -(6-methoxy-1-naphthyl)-butyric acid was obtained in excellent yield by dehydration with sulfur. Kon and F. C. J. Ruzicka, *J. Chem. Soc.*, 187 (1936), synthesized 8- and 9-hydroxy-1-ketotetrahydrophenanthrene starting with the β -methoxynaphthylethyl alcohols obtained by the action of ethylene oxide on the Grignard reagents from 1-bromo-4-methoxynaphthalene and 1-bromo-5-methoxynaphthalene. The chains were lengthened by the malonic ester synthesis, the γ -arylbutyric acids

were cyclized, and the ketones demethylated. By application of the standard methods the naphthylethyl alcohols were converted also into 8- and 9-methoxycyclopentenophenanthrene.

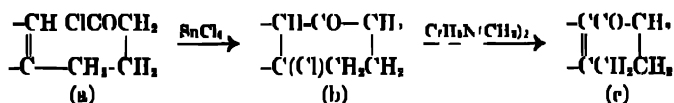
γ -*m*-Methoxyphenylbutyric Acid (p. 222). In preparing further quantities of the acid, R. Robinson and J. Walker, *J. Chem. Soc.*, 192, 747 (1936), introduced certain technical variations in the synthesis (b) from *m*-methoxybenzaldehyde (condensation of the aldehyde with methyl acetate or with malonic acid in pyridine, introduction of an ester group by a Grignard reaction with methyl chloroformate). Another method of preparation was investigated by Chuang and Huang, *Ber.*, 69, 1505 (1936), and by Martin, *J. Am. Chem. Soc.*, 58, 1438 (1936). β -Benzoylpropionic acid was converted through the *m*-nitro derivative, the amine, and the hydroxy compound into Thompson's β -*m*-methoxybenzoyl propionic acid (XIII) and this was reduced by the Clemmensen method. The reactions proceed satisfactorily except in the last step. Martin found that considerable resinification occurs in the reduction of any but very small quantities, even when employing toluene (p. 347). No difficulty was encountered in the preparation of γ -3-methoxy-4-methylphenylbutyric acid by the same method.

Robinson-Rapson Synthesis (pp. 222-223). The structure XVI assigned to the condensation product has been confirmed by X-ray crystallographic analysis and by the observation that the compound on hydrogenation yields a tetrahydro derivative of alcoholic function, as shown by acetylation [Crowfoot, Rapson and R. Robinson, *J. Chem. Soc.*, 757 (1936)]. Further evidence that the condensation proceeds as indicated is furnished by the successful use of the method in the synthesis of known hydrocarbons. From α -tetralone and acetylcyclohexene, Peak and R. Robinson, *ibid.*, 759 (1936), obtained in excellent yield a mixture of three isomeric ketodecalhydrochrysenes. Two of these are believed to be stereoisomers and the third is regarded as having the double bond in a different location. The nature of the ring system was established by conversion to chrysene. Hawthorne and R. Robinson, *ibid.*, 763 (1936), found that the condensation of α -tetralone with acetylcyclopentene proceeds less smoothly, and only one ketotetrahydrocyclopentanophenanthrene was isolated. Peak, R. Robinson and J. Walker, *ibid.*, 752 (1936), made a preliminary study of other possible variations of the Knoevenagel reaction for synthetic purposes. Rapson, *ibid.*, 1626 (1936), investigated the condensation of 2-carbethoxycyclohexanone and the corresponding cyclopentanone derivative with unsaturated methyl ketones.

Robinson-Schlittler Synthesis (p. 223). As an alternate route to the intermediate diketone XIX, Hewett, *J. Chem. Soc.*, 50 (1936), investi-

gated the condensation of β -*m*-methoxyphenylethyl bromide with the potassium derivative of dihydroresorcinol. Although some of the desired compound was obtained, the chief product was that of O-alkylation and the process was declared unsuitable for preparative purposes.

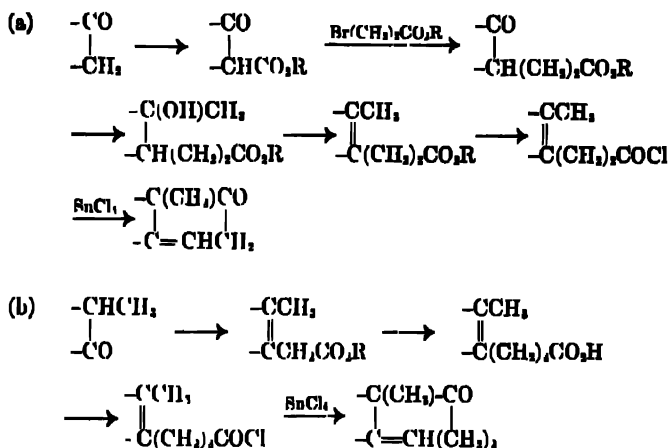
As a further step toward the hormone type of structure, R. Robinson and J. Walker, *ibid.*, 192 (1936), succeeded in preparing the 2-methyl derivative of 7-methoxy-1-ketohexahydrophenanthrene, XX. This was accomplished by employing ethyl α -acetyl- α' -methylglutarate in the first step of the synthesis. The cyclization of the methyl derivative of the diketone XIX might occur in two ways, but by varying the last two stages of the synthesis it was found that the ring closure involves the less hindered carbonyl group, as anticipated. The methylated keto ester corresponding to XVIII was cyclized with sulfuric acid at -15° to the 1-substituted Δ^1 -dihydronaphthalene derivative. In order to close the third ring, use was made of an adaptation of the Darzens reaction [*Compt. rend.*, 150, 707 (1910)] first used for the synthesis of cyclic compounds by Cook and Lawrence, *J. Chem. Soc.*, 1637 (1935). This consists in the intramolecular condensation of an unsaturated acid chloride (a) to a chloroketone (b),



followed by the elimination of hydrogen chloride with dimethylaniline to give an unsaturated ketone (c). The final product obtained in this way was identical with the substance prepared by the first synthesis and consequently the location of the methyl group at the 2-position is established.

For the application of another plan of synthesis the saturated ketone corresponding to XX was required. Having no success in attempting to effect a direct saturation of the ethylenic linkage, R. Robinson and J. Walker, *ibid.*, 747 (1936), reduced XX to a saturated alcohol which was then oxidized to the ketone. The crystalline 7-methoxy-1-ketooctahydrophenanthrene isolated from the resulting mixture appeared from X-ray crystallographic analysis to have the trans configuration. When the oxidation was not conducted under very mild conditions, the hydronaphthalene nucleus was aromatized in the course of the reaction and the ketone of Butenandt and Schramm (ether of XI, p. 221) was obtained. Starting with the saturated trans ketone, a carbethoxy group was introduced at the 2-position by condensation with ethyl oxalate and decomposition of the oxalyl derivative, and a methyl group was then introduced at position 2 by alkylation of the β -keto ester.

Chuang's Syntheses (addition to p. 223). The Darzens reaction, introduced as a method of cyclization by Cook and Lawrence (see above), was employed by Chuang, Tien and Ma, *Ber.*, 69, 1494 (1936), in the elaboration of two interesting general methods for the synthesis of polycyclic compounds containing angular methyl groups. The two schemes are indicated by means of partial formulas. Isomers can arise in the Darzens cyclization and only one possible location of the new double bond is shown.



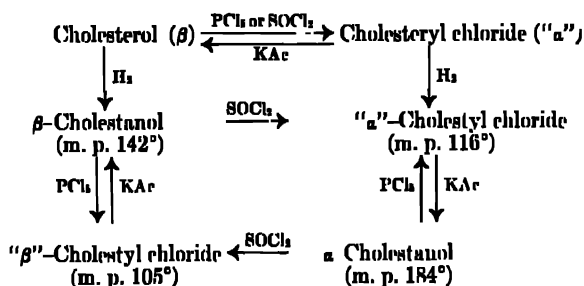
Chuang, Tien and Ma prepared angular methyl derivatives of α -hydrindanone and of α -octalone by methods (a) and (b), respectively. The 9-methyloctalins ($\Delta^{4,10}$ or $\Delta^{7,10}$) was not affected by treatment with selenium at 400°, but yielded naphthalene when the temperature was raised to 450°.

Natelson-Gottfried Synthesis (addition to p. 223). Natelson and Gottfried, *J. Am. Chem. Soc.*, 58, 1432 (1936), explored an interesting plan of synthesis which involves the construction of a molecule having all features of the structure of 3'-keto-1,2-cyclopentenophenanthrene except the diphenyl linkage between rings A and C. This linkage might then be established by a suitable process of cyclization. Furthermore, by suitable modification of the tricyclic compound it might be possible to approach the type of structure found in vitamin D. Natelson and Gottfried developed an ingenious process for the synthesis of the desired 4-(β -phenylethyl)-hydrindone-1 and are investigating the above possibilities.

Male Hormone Activity of Hydrogenation Products of Oestrone (p. 226). In a further investigation of crystalline products of the nuclear hydrogenation of oestrone, (p. 384) and of mixtures, Dirscherl,

J. Kraus and H. E. Voss, *Z. physiol. Chem.*, **241**, 1 (1936), came to the conclusion that crystalline preparations of oestrone from mare's and stallion's urine (but not those from human pregnancy urine) contain an unknown precursor which by hydrogenation is converted into a product with a powerful action on the seminal vesicles but inactive in the comb growth test.

Epimeric Cholestyl Chlorides (p. 230). The information available concerning the chloro compounds obtainable from cholesterol, dihydrocholesterol (β -cholestanol), and *epi*dihydrocholesterol (α -cholestanol) is summarized in the accompanying chart.



The observation that the cholestanols (but not cholesterol) yield different chlorides according as they are treated with phosphorus pentachloride or with thionyl chloride was made by Marker.³⁰

While the configurational relationships of the saturated chloro compounds to the alcohols have not been determined with certainty, Ruzicka³¹ suggested that, since α -cholestanol (*epi*) has a higher melting point than β -cholestanol, the higher melting cholestyl chloride also has the true α (*epi*)-configuration. If this inference proves to be correct, the prefixes " α " and " β " arbitrarily assigned in the literature to the two cholestyl chlorides will correspond to the true configurations at C_3 referred to that of cholesterol. While the difference in melting point is not great, a comparison of the melting points of several pairs of epimers obtained from the chlorides by degradative reactions offering no opportunity for Walden inversion reveals a definite trend in the same direction.

MELTING POINTS OF EPIMERS

α -Cholestyl chloride	116°	β -Cholestyl chloride	105°
3(α)-Chloroallocholanolic acid	176°	3(β)-Chloroallocholanolic acid	196°
Methyl ester	128°	Methyl ester	135°
3(α)-Chloroallonorcholanolic acid	234°	3(β)-Chloroallonorcholanolic acid	213°
Methyl ester	176°	Methyl ester	159°
α -Chloroandrosterone	173°	β -Chlorandrosterone	128°

The data assembled in the table for the acidic degradation products are taken largely from the paper of Barr, Heilbron and Spring, *J. Chem. Soc.*, 737 (1936); the chloroandrosterones have been studied by various workers (pp. 230, 233). In four of the six cases the α -compound melts at a higher temperature than its epimer, and the average increment for the six pairs is $+11^\circ$. This general trend in the melting-point characteristics lends definite weight to Ruzicka's assumption. Unfortunately the optical constants are not available for comparison.

If the configurations are as indicated, the reactions represented in the above chart by vertical arrows all proceed without steric rearrangement and those written horizontally involve Walden inversions. According to this interpretation Walden inversions occur in all the metathetical reactions of the unsaturated compounds (cholesterol and cholesteryl chloride), while all of the transformations of the saturated compounds, with the exception of the reactions of the cholestanols with thionyl chloride, proceed without inversion.

Chloroandrostenone (p. 232). The striking observation that this substance (I) can be converted into compounds of the opposite configuration at C_3 (II and III) finds a close parallel in the transformation of cholesteryl chloride (α) into α -cholestanol, by hydrogenation and hydrolysis, and into cholesterol (β), by hydrolysis (p. 392). Since there are indications that in the sterol series a Walden inversion is more likely to occur in the reactions of unsaturated than of saturated compounds, it is probable that the transformation of chloroandrostenone (I) into androsterone (II) through the saturated ketone proceeds without inversion and that an inversion occurs in the direct hydrolysis to III. Chloroandrostenone therefore probably has the α (*epi*)-configuration at C_3 , a conclusion which agrees with the observation (p. 233) that it yields on hydrogenation a saturated chloroketone (m.p. 173°) which can be characterized as α -chloroandrosterone (p. 392).

Butenandt and Grosse, *Ber.*, 69, 2776 (1936), found that chloroandrostenone can be prepared very conveniently from dehydroisoandrosterone by the method developed by Beynon, Heilbron and Spring for the conversion of cholesterol to cholestyl chloride (p. 364). The *p*-toluenesulfonate of the hydroxy compound yields an abnormal methyl ether on alcoholysis in the presence of potassium acetate, and the methoxyl group is easily replaced by halogen on treatment with acid.

Oxidation of Cholesteryl Acetate Dibromide (p. 234). As one by-product in the preparation of dehydroisoandrosterone by the oxidation of cholesteryl acetate dibromide, a number of investigators^{42, 43, 47} have isolated 3-hydroxy- Δ^5 -cholenic acid. The unsaturated acid was obtained also by Fujii and Matsukawa, *J. Pharm. Soc. Japan*, 56, 433 (1936), and

degraded to 3-hydroxybisanorcholenic acid (II, p. 247). These authors, *ibid.*, 56, 158 (1936), also report the isolation from the mixture of a substance regarded as somewhat impure Δ^6 -pregnenol-3-one-20 (p. 247) as the acetate. Kuwada, *ibid.*, 56, 75 (1936), isolated a product of over-oxidation which was characterized as 3-hydroxy- Δ^6 -aetiobilianic acid by conversion to aetioallobilianic acid (p. 331) [Kuwada and Miyasaka, *ibid.*, 56, 631 (1936)].

Bio-assay of Hormone Preparations (p. 236). Details and variations of the test method are discussed by Gallagher and F. C. Koch, *J. Pharmacol.*, 55, 97 (1935), Ramirez and Rivero, *J. Am. Pharm. Assoc.*, 25, 99 (1936), and Dessau, *Acta Brevia Neerland.*, 5, 139 (1935). Procedures for the extraction of hormones from urine for biological assay are described by Callow, *Lancet*, II, 565 (1936), and by Gallagher, F. C. Koch and Dorfman, *Proc. Exptl. Biol. Med.*, 33, 440 (1935).

Crystallizates from Testicular Extracts (p. 240). The hormone isolated by Ogata and Hirano⁶⁰ has been compared with androstanedione by Hirano, *J. Pharm. Soc. Japan*, 56, 717 (1936), and found to be quite different from this compound. In a further investigation, Hirano isolated four new substances in amounts varying from 50 mg. to 200 mg. from 50 kg. of hog testes. Substance A, m.p. 258-264°, is assigned the formula $C_{21}H_{32}O_3$ and called **testalolone**. The compound forms an insoluble digitonide, a monobenzoate, and a dioxime; it has the reducing properties of an aldehyde, and the ring system is saturated. From these and other observations, and in analogy with known substances, Hirano provisionally suggests that testalolone is a derivative of 3-hydroxy-androstane with the group COCHO as a substituent at C_{17} . Substance B, m.p. 95-96°, $C_{18}H_{26}O_3$, has one free hydroxyl group and yields palmitic acid on hydrolysis. The other hydrolysis product gives formaldehyde on treatment with lead tetraacetate. Hirano provisionally regards the new substance as a monopalmitate of propanediol-1,2 and is investigating its possible relationship to Laqueur's X-substance (p. 396). Substance C, m.p. 65-66°, $C_{10}H_{16}O_3$, is called **testriol** and considered to be an open-chain, triatomic alcohol, probably $(CH_3)_2C(OH) \cdot C_{14}H_{24}CH(OH)CH_2OH$. Substance D, m.p. 219-224°, is probably a C_{23} - or C_{24} -compound containing three atoms of oxygen.

Activity of Androsterone and Testosterone Derivatives (p. 240). Much interest has been shown in the preparation and bio-assay of 17-alkyl derivatives of testosterone. Ruzicka^{68, 69} found that the methyl derivative can be obtained readily from dehydroisoandrosterone by a process even simpler than that employed for the preparation of testosterone itself (p. 239). The unsaturated hydroxyketone (I, p. 239) is treated with excess Grignard reagent and the resulting diol is oxidized in the

form of the dibromide. On debromination, the double bond migrates from the 5,6- to the 4,5-position and 17-methyltestosterone (m.p. 164°) is obtained. Fujii and Matsukawa, *J. Pharm. Soc. Japan*, 55, 1333 (1935), reported the independent preparation of the compound by the same method. According to most reports, 17-methyltestosterone is about equal in activity to testosterone in the caoon test and has a slightly greater activity in the rat test.

While the introduction of a methyl group seems to enhance somewhat the physiological actions of the natural hormone, an ethyl group has the opposite effect and results in a decided loss in activity. The preparation of 17-ethyltestosterone (m.p. 139°) was undertaken by both Butenandt and Ruzicka, and certain difficulties were encountered in applying the standard method [Butenandt, Cobler and Josef Schmidt, *Ber.*, 69, 448 (1936); Butenandt and Schmidt-Thomé, *ibid.*, 69, 882 (1936); Ruzicka and Rosenberg, *Helv. Chim. Acta*, 19, 357 (1936)]. It eventually was recognized in both laboratories that some reduction of the 17-carbonyl group occurs in the course of the reaction of dehydroisoandrosterone (and similar ketones) with ethylmagnesium iodide. No reduction was observed when using the methyl Grignard reagent, while with propylmagnesium iodide reduction predominated over addition. The reduction by the Grignard reagent follows chiefly the same steric course as catalytic hydrogenation, although Ruzicka at first was misled on this point by the difficulty of isolating the unmethylated diol in a pure condition. In the final stage of the process, Butenandt obtained a compound which at first was regarded as the true (Δ^4) 17-ethyltestosterone but which was later found to be the Δ^5 -isomer. The conditions of the original debromination evidently were not such as to promote the usual bond migration, but it was found that the Δ^5 -compound is isomerized easily by acids (compare p 359).

Applying the standard synthetic method, Fujii and Matsukawa, *J. Pharm. Soc. Japan*, 56, 225 (1936), prepared 17-benzyltestosterone (m.p. 225°), while Kuwada and Yago, *ibid.*, 56, 625 (1936) prepared 17-vinyltestosterone (m.p. 170°) and 17-allyltestosterone (m.p. 137°). Both the vinyl and the allyl compound were found to be about one-third as active as testosterone in the rat test.

From a by-product obtained in the hydrogenation of an intermediate in the preparation of a quantity of testosterone, Ruzicka and Kāgi, *Helv. Chim. Acta*, 19, 842 (1936), were able to prepare an isomer of testosterone having the opposite configuration at C₁₇. It was found that inversion at this position results in a great loss in hormonal activity. Butenandt and Hanisch, *Ber.*, 69, 2773 (1936), undertook the preparation of the Δ^5 -isomer of testosterone and succeeded in obtaining the compound

in the form of the acetate by the process of debromination in neutral solution (p. 359). It was not possible, however, to hydrolyze the acetate without rearrangement of the double linkage. Δ^5 -Androst-enolone acetate was found to be only about one-half as active as testosterone acetate in both the capon and rat tests. Ruzicka and Wettstein, *Helv. Chim. Acta*, **19**, 1141 (1936), prepared a series of esters of testosterone and found that, as the hydrocarbon residue of the ester group is increased in size, the activity measured by the comb growth test falls off while that measured in the rat test increases. The enol diacetate of testosterone is comparable with the monoacetate in androgenic activity and it gives no response in the Allen-Doisy test [Ruzicka and W. H. Fischer, *ibid.*, **19**, 1371 (1936)]. 17-Aminoandrostanol-3(α) (m.p. 187-188°) was prepared by the reduction of androsterone oxime by Ruzicka and Goldberg, *ibid.*, **19**, 107 (1936), and by Marker, *J. Am. Chem. Soc.*, **58**, 480 (1936). Tests showed that the replacement of the hydroxyl group at C₁₇ by an amino group results in greatly reduced activity.

The relationship between structure and physiological activity among compounds of the androsterone group has been reviewed recently by Tscherning, *Angew. Chem.*, **49**, 11 (1936), and by Ruzicka, *ibid.*, **49**, 28 (1936); *Helv. Chim. Acta*, **19**, E89 (1936). The biological activities of a number of the compounds have been investigated further by Deaneely and Parkes, *Biochem. J.*, **30**, 291 (1936), Korenchevsky, Dennison and Brovsin, *ibid.*, **30**, 558 (1936), and Korenchevsky and Dennison, *ibid.*, **30**, 1514 (1936).

Activation of Testosterone by the X-Substance (addition to p. 240). It was discovered by Laqueur and co-workers⁶⁰ that a substance present in testes and called the X-substance is capable of enhancing greatly the activity of testosterone on seminal vesicles and prostates of castrated rats. The substance is inert when injected alone, but when injected together with testosterone the activity of the latter is amplified from five to ten times. The X-substance serves also as an activator of androsterone and androstanediol [Dingemans and Polak, *Acta Brevia Neerland.*, **5**, 179 (1935)]. Laqueur characterized the substance as fat-soluble and acidic, and Miescher, Wettstein and Tschopp, *Chemistry and Industry*, **55**, 238 (1936), found that certain higher fatty acids share the property of acting as accessory substances. Palmitic acid is one of the most active and exhibits all the actions of the X-substance. Ricin-elaidic, stearic, and elaidic acids showed progressively decreasing potency. Since the fatty oils commonly used as solvents in the bio-assay of male hormones probably contain varying amounts of activators in the form of free fatty acids, the problem of standardization is clearly complicated by this factor. The effectiveness of a hormone varies both with the

nature and the amount of the solvent used [Deanesly and Parkes, *Lancet*, I, 837 (1936)].

Oestrogenic Activity of Compounds of the Androsterone Group (addition to p. 240). Following the discovery that androstenedione and dehydroisoandrosterone possess some oestrogenic activity (p. 236), Butenandt, *Naturwissenschaften*, 24, 15 (1936), found that androstenediol (II, p. 239) combines to a marked degree the physiological properties of male and female hormones. The substance induces oestrus in castrated female mice and promotes comb growth in capons, and it causes the premature development of female as well as male infantile rats. Butenandt found the substance to be active in a total dosage of 4×0.2 mg. in the Allen-Doisy test with mice (1-2 days oestrus). Similar observations concerning androstenediol and its 17-methyl derivative were reported by Tschopp, *Arch. intern. pharmacodynamic*, 52, 381 (1936) [see also Deanesly and Parkes, *Brit. Med. J.*, 1, 257 (1936)]. Butenandt and Dannenberg, *Ber.*, 69, 1158 (1936), prepared Δ^1 -androstenedione from 2-bromoandrostanedione-3,17 (compare pp. 249-251) and found that, while the compound has no male hormonal actions, it gives a positive response in the Allen-Doisy test in 4×0.5 mg. dosage. On comparing this substance with Δ^4 -androstenedione, a compound comparable in activity with the male hormone androsterone, it is striking that the transposition of the double bond from the 4,5- to the 1,2-position results in a compound having the actions of a follicular hormone. Still other oestrogenic substances of the androsterone group were discovered by Butenandt and B. Riegel, *ibid.*, 69, 1163 (1936). 6-Ketotestosterone, obtained as the acetate by oxidizing the 17-monoacetate of Δ^5 -androstenediol-3,17 with chromic anhydride, gave a positive reaction in the Allen-Doisy test in 4×0.5 mg. dosage. Δ^4 -Androstenetrione-3,6,17 has about the same oestrogenic activity.

Marker, O. Kamm, Oakwood and Laucius, *J. Am. Chem. Soc.*, 58, 1948 (1936), found that a mixture of the epimeric 3-carbethoxyandrosteroes gave an oestrous response in rats when administered in quantities of 5-10 γ , although no activity in the comb growth test was observed in doses of 2 mg. The mixture of free acids was much less active than the esters. The material was prepared by oxidizing a mixture of epimeric 3-carbomethoxycholestanes obtained by the carbonation of cholesteryl-magnesium chloride, followed by hydrogenation and esterification [Marker, Oakwood and Crooks, *ibid.*, 58, 481 (1936)].

The preparation of a compound of remarkable potency as both a male and female hormone has been reported by Fujii and Matsukawa, *J. Pharm. Soc. Japan*, 56, 543 (1936). Dehydroisoandrosterone, treated with dry hydrogen chloride, gave 5-chloroisoandrosterone (Ruzicka⁶⁸),

from which there was obtained by oxidation and bromination a substance described as 2-bromo-5-chloroandrostanedione. On treatment with potassium acetate and acetic acid this yielded a doubly unsaturated diketone, m.p. 168°, regarded as Δ^1 -dehydro- Δ^4 -androstenedione-3,17. The capon unit reported for the compound is about equal to that of androsterone, and it was found that the injection of 0.5 mg. of material in four doses into a castrated mouse gave a positive Allen-Doisy test.

Stereochemical Nomenclature (addition to p. 240). According to the proposal of Ruzicka (p. 116), the steric arrangement of a substituent at the 3-position is indicated by a prefix, *cis* or *trans*, to express the conventionally assumed relationship of the group to the hydrogen atom at C₅. In this system androsterone (II, p. 228) and isoandrosterone (I, p. 228) are 3-*cis* and 3-*trans* compounds, respectively. In the case of unsaturated sterols having no hydrogen at C₅, the arrangement of the group at C₃ is referred to that of the corresponding product of hydrogenation. Dehydroisoandrosterone, for example, is designated *trans* because in the hydrogenation product the substituents at 3 and 5 are assumed by convention to have this relationship (p. 233, note 39). Although Ruzicka's proposal has been accepted by a number of authors, Schoenheimer and Evans, *J. Biol. Chem.*, 114, 567 (1936), have criticized the system on the ground that the hydrogen atom at C₅ is not a very satisfactory point of reference even in the saturated sterols, since it is not fixed for all known compounds. The two broad classes of steroids derived from cholestane and from coprostane have opposite configurations at C₅, and an awkward situation arises in the case of isomers such as dihydrocholesterol and coprosterol (p. 116). Considering the assumed relationship of the groups at C₃ and C₅, the substances are designated 3-*trans* and 3-*cis*, respectively, and yet the arrangement of the hydroxyl group at C₃ in each case corresponds to that of cholesterol and both compounds are precipitable with digitonin. In order to avoid this difficulty, Schoenheimer and Evans proposed that the steric arrangement of the hydroxyl group should be described in terms of its relationship to the methyl group at C₁₀ [see also Lettré, *Ber.*, 68, 766 (1935)]. According to this system, dihydrocholesterol and coprosterol are both 3-*cis* compounds, while their epimers are designated 3-*trans*. This scheme has the advantage of affording a ready distinction, at least with the commoner substances, between sterols which form insoluble digitonides and those which do not. The choice of the C₁₀-methyl group as a fixed point of reference, however, is not without objection. Although the stereochemical relationship of this group to the rest of the molecule is the same in a great many of the known sterols and sterol derivatives, there are definite indications in the work of Dimroth on lumisterol (p. 375) that in the case of the irradiation

products of ergosterol the configuration at C_{10} is the opposite of that of the natural sterols. The new proposal therefore does not avoid entirely one serious difficulty of Ruzicka's system even as applied to such compounds as are known at the present time, and provision for new compounds is definitely lacking. It is questionable if any single center of asymmetry in the molecule can provide a rational reference point for defining the configurations at other centers.

A further serious objection to the above proposals is that the steric relationship of the C_3 -hydroxyl to the C_5 -hydrogen atom or to the methyl group at C_{10} is still uncertain. Ruzicka's assumption that the substituents at C_3 and C_5 in dihydrocholesterol are located on opposite sides of the general plane of the rings may be correct, but it is by no means established. As a provisional convention for purposes of formulation until the matter can be settled, his plan of representing the one valence by a full line and the other by a dotted line is very useful, but the arbitrary and uncertain nature of the assumption should not be lost sight of, and it appears definitely inadvisable to incorporate into the system of nomenclature an assumption which may prove to be incorrect. Even when secure evidence of the configurations becomes available it is questionable if the relationship between centers of asymmetry such as C_3 and C_{10} should be defined by the terms *cis* and *trans*, for these prefixes most commonly refer to adjacent centers and bear definite implications regarding steric hindrance and the opportunity for ring formation. Probably a new set of prefixes, and one capable of defining the relationship of each asymmetric carbon atom to the molecule as a whole, would be more appropriate.

As for the arrangement of the characteristic hydroxyl group of the sterols, evidently it is the relationship of this group to the central plane of the ring system which is of prime importance, and this relationship, although unknown, can be defined accurately and adequately by reference to cholesterol. The configuration at C_3 common to cholesterol, dihydrocholesterol, coprosterol, ergosterol and similar steroids is appropriately designated where necessary by the classical prefix β , while the epimers of these substances are 3(α)-hydroxy compounds, as are the bile acids and androsterone. No arbitrary assumptions are involved in this nomenclature, the facts regarding precipitability with digitonin find a satisfactory correlation, and the standard convention can be retained in writing formulas until final evidence becomes available. For natural products, the common names usually are entirely adequate, and usually it is possible with the use of the prefixes α and β , and sometimes *iso*, to assign satisfactory names to their derivatives and to related compounds. For purposes of specific reference to the configuration at C_3 ,

androsterone may be called α -androsterone, since it corresponds to α -cholestanol (*epidihydrocholesterol*). The companion substance isolated from urine was named "dehydroandrosterone" at a time when there was no reason to suppose that a Walden inversion occurs in one of two similar hydrolysis reactions (pp. 232-233). Since it is now known that the steric arrangement at C_8 is the opposite of that of androsterone, the original name is recognized as misleading. The substance is derived from the diastereomer of androsterone, and hence in this book it has been called dehydroisoandrosterone. An equally acceptable, and perhaps preferable, name for isoandrosterone is β -androsterone, and dehydroisoandrosterone may be called 3(β)-hydroxy- Δ^5 -androstenone-17, or Δ^5 -androstenol-3(β)-one-17. These names reveal at once the correspondence of the steric arrangement of the hydroxyl group to that of cholesterol. They are as specific as the *cis-trans* designations, and they imply no assumptions concerning the absolute configurations or a special point of reference.

The proposed system represents only a slight extension of the nomenclature currently employed by most authors who have not adopted *cis-trans* prefixes in the naming of compounds, and it retains for a key compound of the steroids the classical name β -cholestanol. For the present no drastic revision of traditional names is called for. It is probable, if not certain, that the so-called α - and β -cholestyl halides actually bear the configurational relationship to α - and β -cholestanol implied in the names fortuitously assigned to the compounds (p. 392). Unless the present indications are not sustained by final evidence, the names can be retained. Where the prefixes α and β have been used in assigning provisional names to isomers of unknown structure, as in the case of the ergosterols (p. 371), any possible confusion will vanish as the structures become fully established. Where there is any possibility of confusion with such arbitrary and provisional prefixes, the letter indicating a configurational relationship can be attached to the number. β -Cholestanol, for example, can be given the more specific name cholestanol-3(β).

If the proposed plan of indicating the configuration at C_1 is adopted, it would seem logical to extend the system by the use of a compound such as dihydrocholesterol as a standard, the configuration of all asymmetric centers in the molecule being assigned the designation β . An example of this usage is given in the following section.

Configuration at C_{17} (addition to p. 240). In accordance with the above principles, the saturated diols from androsterone (α) and from isoandrosterone (β) may be called androstane-3(α),17-diol (androstane-3(α),17-diol) and androstane-3(β),17-diol (isoandrostane-3(β),17-diol). While the diacetate of the 3(β),17-diol on partial hydrolysis yields chiefly the 17-mono-

acetate,⁸⁷ Ruzicka and Goldberg, *Helv. Chim. Acta*, **19**, 99 (1936), found that the diacetate of the epimeric 3(α),17-diol gives the 3-monoacetate. A similar observation was made in the case of the Δ^5 -unsaturated diols. The greater reactivity of the 3(β)-acetoxy group as compared with the 3(α)-group had been observed by Vavon and Jakubowicz, *Bull. soc. chim.*, [4], **53**, 581 (1933), in comparing the esters of β - and α -cholestanol, and the chief significance of the new results is in the comparison which they afford between groups in positions 3 and 17. The reactivity of an acetoxy group at C₁₇ evidently lies between that of the same group in the two epimeric arrangements at C₃. Ruzicka and Goldberg point out that this order of reactivity is understandable only if it is supposed that the 17-acetoxy group occupies a trans position with respect to the methyl group at C₁₃. If the substituents on the adjacent carbons 13 and 17 were present in a cis arrangement, the 17-acetoxy group would be considerably more hindered than a 3-substituent in either the α - or β -configuration and would tend to survive hydrolysis more effectively in either case. The 17-hydroxyl group of the two diols therefore very probably is present in a trans position with respect to the C₁₃-methyl group.

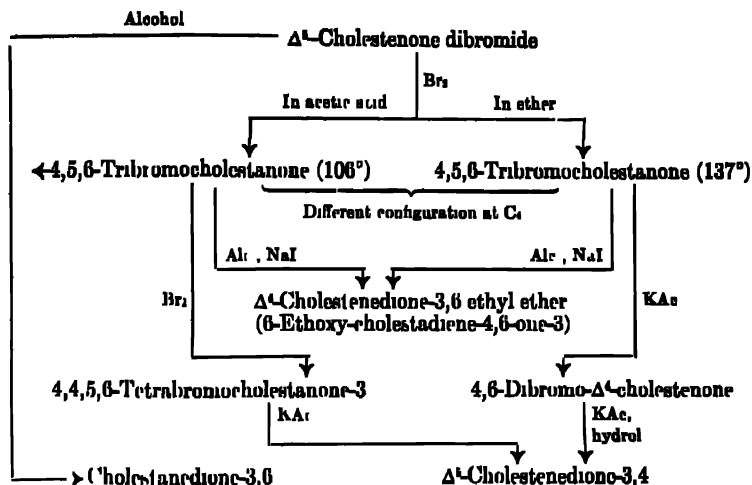
This discovery raises another problem in nomenclature. Although two cis and two trans arrangements are possible for substituents at C₁₃ and C₁₇, it is sufficient at present to take account of only one pair of isomers, since the configuration at C₁₃ is the same in all natural steroids and an inversion at this position has not been observed. The terms cis and trans furthermore are entirely appropriate to the type of isomerism in question, particularly in view of the nature of the evidence upon which the determination of the configuration is based. For these reasons androstenediol and isoandrostenediol are properly assigned the specific names androstenediol-3(α),17(trans) and androstenediol-3(β),17(trans). The term trans defines the steric relationship of the functional group in question to the C₁₃-methyl group, a specific point of reference which is not likely to be mistaken and which appears to be fixed for all steroids. Should isomers be discovered in which an inversion has occurred at C₁₃, it might be desirable to define the configuration at C₁₃ normal to the natural steroids as of the β -type. Androstenediol could be called the 3(α),17 (trans C₁₃ ^{β})-diol, and an inversion at C₁₃ would give the 3(α),17 (cis C₁₃ ^{α})-diol. Such an elaboration, however, is not required at present, and indeed cases are rare in which sufficient evidence is available to warrant the indication in the name of the steric arrangement at C₁₇. Where an inversion at C₁₇ has been observed but where evidence of the configurations is lacking, the abnormal isomer is adequately distinguished from the normal compound by the prefix iso (see, for example, isoallopregnanol-3-one-20, p. 249).

Methods of Bio-assay (pp. 241-242). From a comparison of the Corner-Allen and Clauberg methods of assay, it is reported that the accuracy of the former test can be approached by the Clauberg test only if many animals are used and group averages taken. L. E. Young, *Proc. Soc. Exptl. Biol. Med.*, 34, 96 (1936).

The Two Forms of the Corpus Luteum Hormone (p. 243). While Fels, *Zentr. Gynäkol.*, 59, 2420 (1935), has reiterated the claim that the two compounds differ in their biological actions and are structural isomers, Hohlweg and Josef Schmidt, *Klin. Wochschr.*, 15, 265 (1936), support the view that they are polymorphic crystalline modifications of the same substance and are indistinguishable in their biological effect.

Isolation of Progesterone (p. 245). In later work, Butenandt and U. Westphal, *Ber.*, 69, 443 (1936), developed a more convenient method for the separation of progesterone and the inactive hydroxyketone (allopregnanolone) which accompanies it. On treating the mixture with chlorosulfonic acid the companion substance alone reacts, and the resulting sulfuric acid ester is separated easily as the sparingly soluble sodium salt (ROSO_3Na). The hydroxyketone is subsequently recovered by acid hydrolysis. This convenient method of separation greatly facilitates the purification of both compounds, and no other companion substances were discovered in the extracts. W. M. Allen and Goetsch, *J. Biol. Chem.*, 116, 653 (1936), have described an improved procedure for the extraction of progesterone from pig ovaries whereby 25% of the hormone present in the tissue may be recovered in a pure state.

Bromination of Δ^5 -Cholestenone Dibromide (p. 250). The action of bromine on Δ^5 -cholestenone dibromide, the oxidation product of cholesterol dibromide, has been studied extensively by Inhoffen, *Ber.*, 69, 1134, 1702, 2141 (1936), and by Butenandt and Schramm, *ibid*, 69, 2289 (1936). As monobromination occurs at position 4 rather than 2 it is assumed that the dibromide is of the coprostane series (A/B:ris). A striking observation is that the bromine atom introduced at C_4 assumes different steric arrangements when the bromination is conducted in glacial acetic acid (Butenandt) or in ether (Inhoffen). A few of the many interesting transformations noted in the course of characterizing the isomeric tribromo ketones are indicated in the chart on the opposite page. Bromination of the lower melting tribromo ketone results in the introduction of an additional atom of bromine at the 4-position, and the resulting tetrabromide on treatment with potassium acetate yields Δ^5 -cholestenedione-3,4, a compound which can be obtained also from the higher melting tribromo ketone and which is capable of functioning in an enolic form. Both tribromo ketones are converted by alcohol and sodium iodide into an enol ether of Δ^4 -cholestenedione-3,6 which



Windaus, *Ber.*, 39, 2249 (1906), had obtained by warming the unsaturated diketone with alcoholic hydrochloric acid. The conversion of Δ^5 -cholestenone dibromide into cholestanedione-3,6 with boiling alcohol was observed by Urushibara and Ando, *Bull. Chem. Soc. Japan*, 11, 434 (1936), Ruzicka, Bosshard, W. H. Fisher and Wirz, *Helv. Chim. Acta*, 19, 1147 (1936), and Fujii and Matsukawa, *J. Pharm. Soc. Japan*, 56, 642 (1936). The reactions often proceed through various intermediate stages, and interesting interpretations involving assumed or established additions, eliminations, hydrolyses, and allylic shifts are suggested in the original articles. Further observations concerning polybromo derivatives of cholestenone, cholestanone, and coprostanone are reported by Ruzicka (*loc. cit.*) and by Butenandt, Schramm, Wolff and Kudszus, *Ber.*, 69, 2779 (1936). Dane, Wang and Schulte, *Z. physiol. Chem.*, 245, 80 (1936), also investigated the bromination of cholestenone (and of 3-ketocholanic acid) and introduced a useful new method for effecting the elimination of hydrogen bromide. 6-Bromo- Δ^4 -cholestenone was converted into $\Delta^4,6$ -cholestadienone by the action of silver nitrate in pyridine solution at room temperature. The interesting 3-keto- Δ^4 -cholenic acid (pp. 255, 370) was prepared by these investigators through 3-keto-4-bromocholanic acid.

In the course of his work, Inhoffen observed that cholestenone (Δ^4) is converted by bromine in the presence of hydrogen bromide into 4,6-dibromo- Δ^4 -cholestenone, a reaction which he interpreted as proceeding through the enol form, cholestadiene-3,5-ol-3, produced by the isomerization of cholestenone under the catalytic influence of hydrogen bromide. This view was substantiated in an investigation of the bromination of a

cholestenone enol acetate first prepared by Ruzicka and W. H. Fischer, *Helv. Chim. Acta*, 19, 806 (1936), and formulated as a $\Delta^{2,4}$ - or $\Delta^{1,3}$ -compound. Inhoffen's observations point to the $\Delta^{3,5}$ -structure.

Epiallopregnanol-3-one-20 (addition to p. 250). This substance, which differs from *allopregnanol-3-one-20* (formula XV, p. 249) only in the steric arrangement of the hydroxyl group at C_3 , was isolated from the sterol fraction of 10,000 gallons of human pregnancy urine by Marker, O. Kamm and McGrew, *J. Am. Chem. Soc.*, paper IX, in press. The carbinols were separated from non-carbinol material as the sodium salts of the half-esters of phthalic acid, and the ketonic fraction was separated with the use of Girard's reagent. The *epiallopregnanol-3-one-20* isolated amounted to 1-2 mg. per gallon of urine. The compound melts at 162-164°, it is not precipitable with digitonin, and it yields *allopregnanedione* on oxidation. The new substance occupies an interesting place in the genetic series of substances listed on page 252. As a probable reduction product of the unsaturated ketone progesterone, it is comparable with androsterone with regard to the configuration at C_3 , and has the configuration at C_5 of *allopregnanol-3-one-20*.

Correlation of Progesterone with Androsterone (addition to p. 250). By the conversion of *allopregnanediol*, a reduction product of progesterone, into androstanedione, an oxidation product of androsterone, Marker, O. Kamm, D. M. Jones and Oakwood, *J. Am. Chem. Soc.*, paper VIII, in press, established a relationship between the two hormones.

Specificity of Progesterone (pp. 250-251). Like the Δ^1 -isomer (II), Δ^5 -pregnenedione-3,20 has no progestational activity. It is remarkable that, although the Δ^5 -isomer can be isomerized to progesterone with great ease under the influence of acid catalysts, this change apparently does not occur in the organism under the conditions of the test. The β,γ -unsaturated isomer of progesterone was prepared by debromination of the dibromide in neutral solution (compare p. 359). U. Westphal and Schmidt-Thomé, *Ber.*, 69, 889 (1936). [For a review, see U. Westphal, *Ergeb. Physiol. exp. Pharmacol.*, 37, 295 (1935)].

While many compounds closely related to progesterone in structure do not appear to share the specific physiological properties of the hormone, Klein and Parkes, *Chemistry and Industry*, 55, 236 (1936), reported that certain of the androgenic compounds prepared by Ruzicka elicit typical progestational proliferation of the endometrium. Testosterone itself gave a partial proliferation in a dose of 20 mg. According to a citation of Ruzicka and Rosenberg, *Helv. Chim. Acta*, 19, 357 (1936), 17-methyltestosterone is the most active compound of the group, and in a dosage of 7 mg. has an action equal to that of 1 mg. of progesterone. Progesterone, on the other hand, has no effect on the comb growth of

capons [Albrieux, Buño, Engel and Morató-Manaro, *Klin. Wochschr.*, 15, 206 (1936)].

Origin of the Oestrogenic Hormones (p. 255). In an attempt to test the hypothesis that the oestrogenic agents present in the organism result from the degradation of cholesterol, Rondoni, Carminati and Corbellini, *Z. physiol. Chem.*, 241, 71 (1936), allowed mixtures of cholesterol (10 g.) and minced liver (200 g.) to autolyze for several weeks at temperatures from 37 to 53°. In tests made with benzene extracts, nearly all of the mixtures were found to have oestrogenic activity. H. E. Voss and Rabald, *ibid.*, 245, 76 (1936), however, reported that in conducting similar experiments they had found the commercial cholesterol employed to have sufficient oestrogenic activity to account for the positive results obtained. [See also Rondoni, *ibid.*, 245, 78 (1936)].

Origin and Metabolism of Cholesterol (p. 255). Evidence that coprostanone is not an intermediate in the hypothetical conversion of cholesterol into bile acids in the animal organism has been presented by Schoenheimer, Rittenberg, Berg and Rousselot, *J. Biol. Chem.*, 115, 635 (1936). Dogs with bile fistulas were injected intravenously with an emulsion of coprostanone-4,5d₂ (p. 123) and the fistula bile subsequently collected was examined for the presence of deuterium. It was found that although some of the injected material had passed through the liver this organ had not utilized the sterol derivative in the formation of cholic acid, the cholic acid isolated containing no detectable amount of deuterium. Similar experiments with unsaturated ketones and keto acids would be of the greatest interest.

In considering the origin of cholesterol in the body, Windaus [quoted by H. Lettré and H. H. Inhoffen, "Über Sterine, Gallensäuren, u. verw. Naturstoffe," p. 103 (1936)] has offered the conjecture that the four-ring system comes from oleic acid, through civetone, the angular methyl groups being supplied in a reaction with formaldehyde. A relation between sterol synthesis and fat metabolism in plants is suggested by the observation of MacLachlan, *J. Biol. Chem.*, 113, 197 (1936), that as germination in soy beans progresses there is a diminution in the total fats present and an increase in the sterol content. In studying fat metabolism in the animal organism, Schoenheimer and Rittenberg, *ibid.*, 114, 381 (1936), found with the use of heavy water that in living mice fatty acids are continuously destroyed and replaced, even in fat depots, the acids almost certainly being formed by synthesis from carbohydrates of the diet. They also demonstrated experimentally that desaturation of fatty acids can occur in the living organism [*Idem, ibid.*, 113, 505 (1936)].

Anchel and Schoenheimer, *J. Biol. Chem.*, 114, 539 (1936), note that the presence of cholestenone as an intermediate product of metabolism is

further suggested by the observation that unsaponifiable material from arteriosclerotic aortas gives an absorption spectrum characteristic of α,β -unsaturated ketones.

Crystalline Substances from the Adrenal Cortex (addition to p. 255). Since the discovery [Rogoff and G. N. Stewart, *J. Am. Med. Assoc.*, **92**, 1569 (1929); Swingle and Pfiffner, *Science*, **71**, 321 (1930)] that the life span of adrenalectomized dogs can be prolonged by administration of an extract of the adrenal cortex, attempts have been made by various investigators to isolate the active hormone or hormones ("cortin"). The dog method of assay unfortunately is subject to serious limitations, which is true also of the adrenalectomized-dog muscle test of Everse and de Fremery, *Acta Brevia Neerland.*, **2**, 152 (1932), and indeed no really satisfactory biological test is as yet available for the evaluation of cortical hormone concentrates. The difficulties inherent in the problem of isolation are greatly augmented by the inadequacy of the biological control, and progress consequently has been slow. Careful fractionations of extracts of adrenal glands have been rewarded by the isolation of a number of crystalline substances, but these all have proved to be physiologically inactive, or at the most of low and questionable activity, when tested in adrenalectomized dogs. The substances nevertheless are of considerable interest, for they are closely related in structure to the male sex hormones.

The chemical studies have been conducted by three groups of investigators, the principal papers being as follows: Pfiffner, Wintersteiner and Vars, *J. Biol. Chem.*, **111**, 585 (1935); Wintersteiner and Pfiffner, *ibid.*, **111**, 599 (1935); **116**, 291 (1936); Reichstein, *Helv. Chim. Acta*, **19**, 29, 223, 402, 979, 1107 (1936); Mason, Myers and Kendall, *J. Biol. Chem.*, **114**, 613 (1936); **116**, 267 (1936). Both Wintersteiner and Reichstein made extensive use of Girard's reagent in separating ketonic from non-ketonic material and in differentiating between ketones of varying degree of reactivity. Several compounds were isolated in independent work in each of the three laboratories, and these are known for the most part by the three sets of serial letters originally assigned to them. While some of the compounds of Wintersteiner and of Reichstein have been identified by direct comparison, and while the correspondence of other substances seems probable, the literature on the subject is at present in a state of some confusion. For this reason and because the formulas and structures are still uncertain in some respects, this review will be limited to the citation of a few of the more important observations.

By suitable methods of oxidation, Reichstein was able to convert his substances A, C, and D into the same saturated diketone, m.p. 178°,

of the probable formula $C_{19}H_{26}O_3$. His compounds E and F similarly yielded the unsaturated diketone adrenosterone (m.p. 224° , $C_{19}H_{24}O_3$), which had been isolated from the cortical extracts. Subsequently adrenosterone was found to yield the saturated diketone (178°) on hydrogenation and consequently all six compounds are now correlated. Since the analytical data and the ketonic character of the substances suggested a relationship to members of the androsterone group, Reichstein investigated the action of the saturated and unsaturated diketones in promoting the comb growth of capons and made the striking discovery that they both possess strong male sex hormone activity, being about one-third and one-fifth as active as androsterone. A similar observation was made by Kendall and co-workers concerning a diketone very similar to adrenosterone and possibly identical with Reichstein's substance. Finally Reichstein proved the suspected relationship to androstanedione and androstenedione by the conversion of the saturated diketone (178°) into androstane and into androstanone-17. One of the carbonyl groups of the diketone is evidently located at C_{17} , and, since a related hydroxyketone of the series is precipitated by digitonin, the second group probably is situated at the 3-position. According to Reichstein's observations, the saturated diketone differs from androstanedione-3,17 by only one additional atom of oxygen, which is present in some unreactive combination. The substance liberated no gas in the Zerewitinoff test and only two carbonyl groups could be detected with certainty. It seems possible that the oxygen atom is present either in oxidic combination, in a carbonyl group occupying a hindered position (C_{11} , C_{13}), or in the form of an unresponsive tertiary hydroxyl group (C_8 , C_9). Adrenosterone is an α,β -unsaturated ketone, probably the Δ^4 -dehydro derivative of the saturated diketone (178°).

Of the other compounds which Reichstein has shown to have the androstane ring system with substituents at 3 and 17 and with an unlocated oxygen atom, two have a saturated ring system hydroxylated at C_3 and two are α,β -unsaturated ketones related to adrenosterone. They are all C_{21} -compounds and have the carbon skeleton characteristic of pregnane or progesterone, a two-carbon group being situated at C_{17} . From present indications the probable structures may be defined further as follows: Reichstein's compound A (Wintersteiner's A, probably Kendall's D), $C_{21}H_{34-36}O_5$, has a saturated nucleus, a C_3 -hydroxyl, an unlocated oxygen, and carries at C_{17} a hydroxyl group and the group $-\text{CH}(\text{OH})\text{CH}_2\text{OH}$. Reichstein's C (Wintersteiner's D) and D are stereoisomers differing from A only in that the side chain is $-\text{COCH}_2\text{OH}$. Reichstein's F (Wintersteiner's F, probably Kendall's E), $C_{21}H_{28-30}O_5$, is an α,β -unsaturated ketone with the carbonyl group at C_3 and the

double bond at C_4-C_5 , and the substituents at C_{17} are hydroxyl and $-\text{COCH}_2\text{OH}$. One oxygen atom is unlocated. From the results of the Zerewitinoff test, Kendall was led to believe that the extra oxygen is present as a tertiary hydroxyl group, but Reichstein has pointed out that the methane liberated may be due to enolization and that a similar result was not obtained with his saturated diketone (178°). The compound E' of Reichstein differs from F only in having the side chain $-\text{CH}(\text{OH})\text{CH}_2\text{OH}$.

Chapter VI

New Glycoside (addition to p. 259). From the seeds of *Strophanthus emini*, Lamb and S. Smith, *J. Chem. Soc.*, 442 (1936), isolated after partial enzymatic hydrolysis the monoside **emicymarin**, $\text{C}_{30}\text{H}_{48}\text{O}_8$. On acid hydrolysis this yielded trianhydroperiplogenin and digitalose.

Oxidation of Strophanthidinic Acid (p. 264.) Elderfield, *J. Biol. Chem.*, 113, 631 (1936), has shown in a reinterpretation of earlier work of Jacobs, *ibid.*, 57, 553 (1923), that when the lactone ring of strophanthidinic acid is saponified and the compound oxidized in alkaline solution with permanganate, the side chain is degraded to $-\text{COCO}_2\text{H}$. The acid group lactonizes with the hydroxyl group at C_{14} and the product of the reaction is an α -keto lactone acid of the formula $\text{C}_{21}\text{H}_{30}\text{O}_8$ (previously $\text{C}_{28}\text{H}_{30}\text{O}_8$).

Zerewitinoff Determination (p. 265). From the results of tests made with a number of aglycone derivatives, Jacobs concluded that the active hydrogen atom of the lactone ring invariably gives one mole of methane with the Grignard reagent [Jacobs and Collins,²¹ Jacobs and Gustus, *J. Biol. Chem.*, 74, 811 (1927)]. Tschesche and Haupt, *Ber.*, 69, 459 (1936), questioned the validity of this generalization, for they observed no evolution of gas in a test with anhydroazarigenin benzoate. They reported also an apparently anomalous result with anhydroconvallotoxinigenin benzoate, but the interpretation in this case is questionable (see p. 417. Jacobs and Elderfield, *ibid.*, 114, 597 (1936), subsequently examined a number of additional substances and found that usually the active hydrogen atom responsible for the Legal reaction liberates a full mole of gas, although in a few cases there was a deficiency of 40-60% in the quantity of methane.

Colorimetric Determination of Molecular Weight (p. 265). Observing that cardiac glycosides and aglycones having an active hydrogen in

the unsaturated lactone ring give with alkaline picric acid solution a characteristic orange-red color, Neumann, *Z. physiol. Chem.*, **240**, 241 (1936), developed a colorimetric method for the quantitative determination of substances of this group. By comparing the color readings of a solution of a cardiac compound with a calibration curve obtained with *k*-strophanthidin, a few milligrams of material can be determined with a high degree of accuracy. The colorimetric method can be used for the determination of the molecular weight of substances having the characteristic β,γ -unsaturated lactone ring.

Dehydration of Epiallocholesterol (p. 282). See p. 362.

Digoxigenin (p. 283). In a paper published almost simultaneously with the first edition of this book, Tschesche and Bohle, *Ber.*, **69**, 793 (1936), on the basis of new experimental evidence, arrived at a formula for digoxigenin identical with that suggested by the author from other considerations. Tschesche and Bohle established the skeletal structure of the aglycone and proved that it belongs to the coprostane series (A/B :cis) by degradation through the anhydro compound, the tetrahydroanhydro compound, and the diketone (m.p. 290°) to a saturated desoxylactone identical with a lactone obtained from digitoxigenin by Windaus and Stein.⁴⁴ On oxidizing the diketone (tetrahydroanhydrodigoxigenone) one of the rings was opened. As the resulting dibasic keto acid gave a diketone on pyrolysis, the ring in question must be ring A and consequently one of the original secondary hydroxyl groups is located in this ring, probably at C₃. Tschesche and Bohle noted that the other secondary hydroxyl group can hardly be in ring A, for it then would be involved in the oxidative opening of this ring. The 6-position also is unlikely, for the dibasic keto acid does not have the properties of a β -keto acid. Positions 15 and 16 can be eliminated because the anhydro linkage of anhydrodigoxigenone would in either case be conjugated with a carbonyl group and the unsaturated diketone would show selective ultraviolet absorption, which is not the case. The anhydro linkage doubtless would migrate to a position of conjugation with a carbonyl group at C₇, and hence this position also is unlikely. Of the remaining positions, 11 and 12, only the former location accounts for the observation that the saturated diketone is isomerized, at least partially, by alkali. A carbonyl group at C₁₁, but not at C₁₂, would be adjacent to an asymmetric carbon carrying a hydrogen atom. In this way Tschesche and Bohle reached the conclusion that the secondary hydroxyl group in question very probably is located at C₁₁, as was inferred by the author from an entirely different set of facts (p. 283).

S. Smith, *J. Chem. Soc.*, 354 (1936), succeeded in isolating two monoanhydro derivatives of digoxigenin similar to the isomeric com-

pounds which he had obtained previously from digitoxigenin [*ibid.*, 1050 (1935)]. The compounds very probably have the expected $\Delta^{14,15}$ and $\Delta^{8,14}$ structures. One isomer, m.p. 182° , was obtained in a pure condition by crystallization of the mixture of substances resulting from the action of dilute acids on digoxigenin. When this isomer was dissolved in concentrated hydrochloric acid at 10° , an unstable chloro compound was formed and separated in a crystalline condition. On treatment with water, the chloro compound was rapidly decomposed and the second anhydrodigoxigenin, m.p. 192° , was formed. The method of effecting the isomerization seems to be generally applicable. Probably the migration of the double bond to an adjacent position is the result of the addition and elimination of hydrogen chloride (compare α - and β -ergosterol, p. 372).

Sarmentogenin (addition to p. 283). Jacobs and Heidelberger⁶ characterized this aglycone from sarmentocymarin (m.p. 137°) as a trihydroxy unsaturated lactone of the composition $C_{23}H_{34}O_5$. They prepared anhydro-, iso-, and dihydro-sarmentogenin by the usual methods, and also found that the aglycone on benzylation in pyridine solution forms a dibenzoate. Jacobs and Heidelberger also reported, without comment, the striking observation that only one of the two groups involved in the benzylation reaction was attacked in an oxidation experiment, the product having the composition of a monoketone and giving a monosemicarbazone. (The composition is sufficiently close to that of a diketone to suggest that the compound may have two carbonyl groups, one of them being inert.) In a recent investigation Tschesche and Bohle, *Ber.*, 69, 2497 (1936) [see also Tschesche, *ibid.*, 68, 423 (1935)], discovered still more definite indications that one of the secondary hydroxyl groups of sarmentogenin occupies a hindered position in the molecule and is similar in this respect to the C_{11} -hydroxyl group of the isomeric aglycone digoxigenin (p. 283). In fact Tschesche and Bohle found that sarmentogenin and digoxigenin have the same structure and differ only in the configuration at C_9 . The correspondence of the skeletal structures was established by the conversion of both substances to the same degradation product. Sarmentogenin yields two anhydro compounds, α (m.p. 208°) and β (m.p. 243°), and the first of these on hydrogenation gave a tetrahydroanhydro compound (α_1 and α_2 mixture). On oxidation there was obtained a diketone (α_1 , m.p. 270°) which was converted into a monoketone (α_1 and α_2) on reduction by the Clemmensen method. The reduction could not be pushed beyond this point and it is now recognized that the inert carbonyl group is located at C_{11} . Evidence that the group eliminated is located at C_9 was found in a separate experiment involving the opening of ring A and the formation of a pyroketone. In

order to remove the oxygen atom at C₁₁, the ketonic group was reduced catalytically and the alcohol was dehydrated to the $\Delta^{9,11}$ -dehydrolactone. On hydrogenation there was obtained a saturated desoxylactone identical with that prepared from both digitoxigenin (Windsaus and Stein ⁴⁴) and digoxigenin (Tschesche and Bohle, see above). It was established that an inversion at C₉ occurs in the course of the degradation of either sarmentogenin or digoxigenin by the preparation from digoxigenin of a $\Delta^{9,11}$ -dehydrolactone identical with that from sarmentogenin (by partial reduction of the 3,11-diketone, hydrogenation to an alcohol, and dehydration). This affords additional evidence of the location of a hydroxyl group at C₁₁ in sarmentogenin, as in digoxigenin, and shows that the two aglycones have opposite configurations at C₉. From the present evidence it is probable, if not entirely certain, that the original C₉-configuration of digoxigenin is retained throughout the degradation, while that of sarmentogenin is retained until the asymmetry at C₉ is destroyed in the formation of the $\Delta^{9,11}$ -dehydrolactone. The compounds having a carbonyl group adjacent to the asymmetric center offer opportunity for inversion, but isomerization does not appear to occur. Since digoxigenin is obtained from plants containing the glycosides digitoxin and gitoxin, and since these companion substances have the configuration at C₇ normal to the sterols and most steroids, Tschesche and Bohle think it probable that digoxigenin has the normal configuration in which rings B and C are linked in the trans arrangement. Sarmentogenin probably represents a rare type of compound having the B/C:cis linkage. This arrangement apparently results in increased steric hindrance at the 11-position. In the digoxigenin series a carbonyl group at C₁₁ is reduced with some difficulty, but with the sarmentogenin compounds the reduction is completely blocked. A corresponding reaction with ketonic reagents occurs in the case of anhydrodigoxigenone, if not with digoxigenone or with tetrahydroanhydrodigoxigenone, but none of the ketones of the sarmentogenin series enter into condensations involving the carbonyl group at C₁₁. Another striking observation is that the saturated alcohol having a lone hydroxyl group at C₁₁ fails to react with benzoyl chloride in pyridine solution. A further point of general interest is the observation of Tschesche and Bohle that the $\Delta^{9,11}$ -dehydrolactone can be hydrogenated in glacial acetic acid solution but not in a neutral alcoholic medium. The double bond extending to the bridge head appears to be comparatively inert.

It is of interest to compare the cardiac activities of the glycosides, sarmentocymarin and digoxin, the aglycones of which differ only in the configuration at C₉. [The results for digoxin are given by K. K. Chen, A. L. Chen and R. C. Anderson, *J. Am. Pharm. Assoc.*, 25, 579 (1936),

while the data for sarmentocymarin were kindly communicated to the author by Dr. K. K. Chen].

	Minimal Systolic Dosage		
	Configuration at C ₉ , B/C	Cat unit, mg. per kg.	Frog dose, mg. per g.
Sarmentocymarin	cis	0.21	0.00545
Digoxin	trans	0.22	0.00250

Digoxin contains three sugar units of a type easily eliminated on hydrolysis (2-desoxy sugar), while sarmentocymarin contains but one monose unit, which is resistant to hydrolysis. If differences in the glycosidic part of the molecule are of minor consequence, the results indicate that an inversion at C₉ does not influence the cardiotonic potency to a very marked extent. On the weight basis the two glycosides are of about the same activity according to the cat unit, while digoxin is about twice as potent as the other substance in the frog test. Since the molecular weight of digoxin is nearly one and one-half times that of sarmentocymarin, a comparison of the substances on the basis of the effective aglycone content would indicate a still greater relative activity for digoxin in the frog test and greater potency in the cat test. The differences, although not great, probably are significant and it is interesting that the substance regarded as having the more normal steric arrangement of rings B and C (trans) is definitely more active than the other compound.

Oxidation of Anhydrodihydrostrophanthidin (pp. 285-286). While the oxidation of anhydrodihydrostrophanthidin (I) with permanganate in alkaline solution results in the hydroxylation of the anhydro linkage at C₁₄-C₁₅ (and oxidation of the aldehydic group), Jacobs and Elderfield, *J. Biol. Chem.*, 113, 611 (1936), found that the reaction takes a different course when permanganate is employed in acetic acid solution. In this case the aldehydic group is attacked as before and an oxido group is introduced between positions 8 and 14. When anhydrodihydrostrophanthidin is oxidized with perbenzoic acid in chloroform solution an 8,14-oxide is similarly produced. The structures of the oxides were established beyond question by a number of transformations, and the same remarkable difference in the course of the oxidation with permanganate in alkaline and in acidic solution was noted with other anhydro aglycones. Since the one reaction indicates that the double bond occupies the 14,15-position while the other points to its location at the 8,14-position, Jacobs and Elderfield concluded that anhydrodihydrostrophanthidin and similar compounds probably exist in solution in both the $\Delta^{14,15}$ and the $\Delta^{8,14}$ forms and that the acidity of the medium

determines their relative abundance or reactivity. Isomeric anhydro compounds of this type have been isolated from usarigenin (p. 284), digitoxigenin (p. 410), digoxigenin (p. 409), and sarmentogenin (p. 410), and it would be of interest to compare the action of oxidizing agents on a pair of such isomers.

Configuration of Strophanthidin (p. 290). Jacobs and Elderfield, *J. Biol. Chem.*, 113, 625 (1936), submitted dihydrostrophanthidin to the cyanohydrin synthesis and in this way transformed the aldehydic group at C₁₀ into $-\text{CH}(\text{OH})\text{COOH}$. The acid is not stable in the free condition but combines with the tertiary hydroxyl group at C₅ to form two stereoisomeric homolactones differing only in the configuration at the new center of asymmetry produced in the synthesis. The nature of the isomerism and the location of the lactone linkage was fully established by suitable transformations. Teschesche and Bohle, *Ber.*, 69, 2443 (1936), have pointed out that a five-membered lactone ring extending between positions 5 and 10 is possible for a *cis* decalin, but not a *trans* decalin, type and consequently that strophanthidin must belong to the copropane series (A/B:*cis*), like digitoxigenin and gitoxigenin. The correlation of periplogenin with strophanthidin shows that this aglycone also has the *cis* linkage between rings A and B, and from evidence to be presented below it is now clear that the same is true of digoxigenin, sarmentogenin, and thevetigenin. Usarigenin is the only aglycone known to have the A/B: *trans* configuration.

Ouabain (pp. 290-295). The interpretation of the structure and reactions of ouabain presented in the first edition has received some support from an observation of Fieser and Newman, *J. Biol. Chem.*, 114, 705 (1936), concerning the acetyltrianhydrolactone (VI, p. 294) from isouabain. As stated on page 294, the validity of the suggested interpretation hinges on the question of whether or not the three nuclear double bonds of the above lactone and of similar degradation products are present in a single, benzenoid nucleus. As the most reliable means of deciding this subtle point in comparable cases is from the absorption spectrum, this method was applied. It was found that the absorption spectrum of the lactone VI is very similar to those of neoergosterol (I, p. 177) and dihydrotrianhydrostrophanthidin (see p. 274). In both of the latter compounds ring B is regarded as aromatic. The absorption maximum of 270 $m\mu$ found for the lactone VI is, according to available evidence, characteristic of a benzenoid ring. Perhaps the most pertinent example of a compound having a triene system distributed in more than one ring is dehydroergosterol (p. 175) which shows an absorption maximum at 320 $m\mu$.

The acetyltrianhydrolactone from iscouabain was found to react only slowly with perbenzoic acid and to consume less reagent than would be expected for a non-benzenoid triene. It was pointed out further that the observation of Jacobs and Bigelow that the lactone can be hydrogenated in acetic acid solution, but resists hydrogenation in neutral solvents, finds a close parallel in Dirscherl's findings concerning the nuclear hydrogenation of oestrone (p. 384).

The conclusion that the acetolysis results in the aromatization of ring B has been confirmed by Tschesche and Haupt, *Ber.*, 70, 43 (1937), by the determination of the absorption spectrum of the lactone $C_{22}H_{28}O_8$ from ouabain. As attempts to aromatize ring A of the methyl ether of this compound by dehydrogenation with platinum were unsuccessful, and because the lactone yields a ketone^{72,78} which, unlike β -tetralone, is not sensitive to acid oxidation, these investigators have questioned the validity of the formulation for ring A suggested by the author for the degradation products. They note further that the tentative formula suggested for ouabain (I, p. 292) should be revised in the light of recent information (see below) by transposing the sugar residue to the 3-position.

Thevetin (addition to p. 299). In 1933 Chen and Chen⁸⁴ isolated the crystalline heart poison thevetin from be-still nuts, the fruit of the plant *Thevetia nerifolia* found in the Hawaiian Islands, South America, and India. Attempts to determine the empirical formula by analysis led to conflicting views,⁸⁴ and unusual difficulties were encountered by Elderfield, *ibid.*, 115, 247 (1936), in attempting to prepare sugar-free derivatives of the glycoside. The whole problem of determining the formula and constitution of thevetin was solved in a series of brilliantly executed experiments by Tschesche, *Ber.*, 69, 2368 (1936). The thoroughly dried glycoside is a hemihydrate of the composition $C_{42}H_{60}O_{18} \cdot 1/2H_2O$, dec. 195°, and a trihydrate is formed on exposure to the air. The genin of thevetin occurs in combination with two molecules of glucose, present in the form of a gentiobiose unit, and one molecule of a methyl ether sugar which probably is digitalose. This sugar has not been identified positively, but it is known to contain one methoxyl group and to be very resistant to hydrolysis. The aglycone thevetigenin ($C_{23}H_{34}O_4$) is known only as the monoanhydro compound, m.p. 218-220°, but the structure is fully established. Thevetigenin is a β,γ -unsaturated lactone (Legal test) having hydroxyl groups at the 3- and 14-positions. That the hydroxyl group at C_{14} is uncombined is shown by the formation of isothevetin, and consequently the entire glycosidic residue is attached at C_3 . The aglycone is an epimer of digitoxigenin (IV, p. 276), from which it differs only in the steric arrangement of the C_5 -

hydroxyl group. Thevetigenin is a 3(β)-hydroxy compound of the type of cholesterol, while digitoxigenin is a 3(α)-compound (*epi*).

In establishing these interesting facts, Tschesche observed that the two glucose residues can be eliminated easily by hydrolysis with acids, and that on treatment with acetic anhydride and zinc chloride the glycoside can be cleaved partially to give octaacetylgentiobiose. The methyl ether sugar is so firmly bound that its elimination invariably is accompanied by secondary changes. By the action of aqueous alcoholic hydrochloric acid on thevetin at the boiling point the two glucose units were removed, but the tertiary hydroxyl group at C₁₄ also was eliminated. Partial hydrolysis of the methyl ether sugar occurs on more prolonged treatment, and anhydrothetvetigenin was isolated from the mixture in small amounts by precipitation with digitonin. In order to obtain sufficient material for investigation, Tschesche hydrogenated the crude product of partial hydrolysis and submitted the more stable tetrahydro product to drastic hydrolysis in order to eliminate the methyl ether sugar completely. The tetrahydroanhydrothetvetigenin so obtained was not identical with the corresponding saturated hydroxylactone from digitoxigenin, but it yielded on oxidation a ketone identical with tetrahydroanhydrodigitoxigenone.

It is now possible to evaluate the importance of stereochemical factors in determining the cardiac activity of typical heart poisons, whereas previously an interpretation of the differences noted was purely speculative (pp. 285, 300-301). Except for the nature of the sugar residue, which probably is of only minor significance in determining the solubilities, digitoxin, thevetin, and uzarin differ only in the steric arrangement at C₃ and C₇ in the aglycone moiety. The results of K. K. Chen, A. L. Chen and R. C. Anderson,⁸³ *J. Am. Pharm. Assoc.*, **25**, 579 (1936), reveal considerable differences in the cardiotonic activities, as indicated in the table. Digitoxin, the most active compound of the series in both the

	Minimal Systolic Dosage		Cat unit, mg. per kg.	Frog dos., mg. per g.
	Configuration C ₁	C ₇ A/B		
Digitoxin	α	cis	0.33	0.0080
Thevetin	β	cis	0.92	0.0045
Uzarin	β	trans	5.08	1.5000

cat and frog tests, is a coprostane derivative of the 3(α)- or *epi*-type. An inversion at C₃ results in only a moderate decrease in activity while an inversion at C₇ as well as at C₃ is attended by an enormous drop in the cardiotonic potency. It is evident that allomerization is of considerably greater importance than epimerization in determining the physiological

properties of both the cardiac drugs and the sex hormones. The previous inference (p. 301) clearly is incorrect.

Convallatoxin (addition to p. 299). The crystalline glycoside convallatoxin (m.p. 238-239°), first isolated by Karrer, *Helv. Chim. Acta*, 12, 506 (1929), from the blossoms of lily of the valley (*Convallaria majalis*), is of particular interest because of its unusually high activity. The substance surpasses all other known heart poisons in this respect. Tschesche and Haupt, *Ber.*, 69, 459 (1936), established for convallatoxin the formula $C_{28}H_{42}O_{10}$ by analyses and by determination of the equivalent weight by titration, and characterized the sugar component in the form of an osazone, very probably that of *l*-rhamnose. As the glycoside gives the Legal test and is isomerized by alkali, it evidently contains the usual β,γ -unsaturated lactone ring and C_{14} -hydroxyl group. In addition to the double bond in the lactone ring, convallatoxinigenin apparently contains another unsaturated linkage which is very resistant to hydrogenation. The glycoside is hydrolyzed only with difficulty and convallatoxinigenin ($C_{28}H_{38}O_8$) itself was not isolated. After benzoylating the crude product of hydrolysis, Tschesche and Haupt succeeded in isolating monoanhydroconvallatoxinigenin as the monobenzoate, m.p. 279-281°. They suggest that the benzoyl group probably replaces the original residue attached to a secondary hydroxyl group at C_8 . In the Zerwitoff test two moles of gas were liberated. Tschesche and Haupt considered this to be an indication of the presence of two free tertiary hydroxyl groups, for in a parallel test with anhydrouzarigenin benzoate they observed no evolution of methane resulting from interaction with the lactone ring. From the character of the hydrogenation curve it was concluded that the anhydro linkage and the double bond of the lactone ring absorb hydrogen readily, but that an unreactive nuclear double bond is still present. This does not appear to be conjugated with the anhydro linkage, for the benzoate shows no selective ultraviolet absorption.

Assuming the skeletal structure to be the same as in the other C_{28} -cardiac aglycones, Tschesche and Haupt suggested tentatively that convallatoxinigenin has a structure similar to that of periplogenin (II, p. 275) but with an additional tertiary hydroxyl group at C_8 and a double bond at the 9,11-position. This location of the double bond probably would account for its inert character (see p. 411) and for the lack of conjugation with the anhydro linkage, for the latter probably is at C_{14} - C_{15} . A hydroxyl group at C_8 would not be under the activating influence of either double bond of the anhydro compound and might well survive the hydrolysis reaction. Tschesche and Haupt expressed the opinion that a tertiary group at C_8 also would persist during the treatment with acid,

but this view has been questioned by Fieser and Newman, *J. Biol. Chem.*, 114, 707 (1936), who pointed out that in analogy with other cases (p. 280) one would expect a group at C₈ to be activated by the adjacent double bonds at the 9,11- and 14,15-positions and to be eliminated easily with the establishment of a conjugated linkage at C₇-C₈. These authors suggested that the hydroxyl group in question may be present as a substituent in the angular methyl group at C₁₀ and that the primary alcoholic group resists benzylation because of steric effects. Since Jacobs and Elderfield, *ibid.*, 113, 611 (1936), recently have shown that in the strophanthidin series the CH₂OH group at C₁₀ can be benzyolated without difficulty, this view is equally untenable.

There seems to be no way of accommodating an inert nuclear double bond and two tertiary hydroxyl groups to the usual skeletal structure. It will be noted that the evidence for the presence of two tertiary hydroxyl groups is confined to the observation that the anhydroaglycone benzoate liberates two moles of methane in the Zerewitinoff test, and it is possible that the observation was misinterpreted. If the reaction follows the course more usual for aglycone derivatives (p. 408), one mole of gas comes from interaction of the Grignard reagent with the active hydrogen atom of the unsaturated lactone ring and the second mole indicates the presence of a single free hydroxyl group. The evidence regarding the inert center of unsaturation also is capable of an interpretation different from that given by Tschesche and Haupt. The presence of this linkage is inferred from the analytical data and from a slow and partial hydrogenation observed with the glycoside and the aglycone benzoate after the more active double bonds had been saturated. The results would be the same if the aglycone contained an aldehydic group at C₁₀ in place of a nuclear ethylenic linkage, for the aldehydic group of strophanthidin is hydrogenated only with great difficulty (p. 265). It is suggested, therefore, that convallatoxinigenin is a compound with a saturated ring system, with an aldehydic group at C₁₀, and with hydroxyl groups at C₈, C₉, and C₁₄. It is equally possible, but less likely in analogy with other cases, that one of the tertiary hydroxyl groups is located at C₉ rather than C₈. The suggested structure is that of strophanthidin, and indeed it seems possible that the two aglycones are identical. Jacobs and Collins, *ibid.*, 59, 713 (1924), report that anhydrostrophanthidin benzoate melts at 287-289°, a value not far from that of the benzoate isolated by Tschesche and Haupt. If not identical, the substances may be stereoisomers.

K. K. Chen, A. L. Chen and R. C. Anderson, *J. Am. Pharm. Assoc.*, 25, 579 (1936), found for convallatoxin the following minimal systolic dosages: 0.08 mg. per kg. cat; 0.00021 mg. per g. frog. For cymarín, a glycoside of strophanthidin, they found the cat and the frog unit to be 0.13 mg.

per kg. and 0.00060 mg. per g., respectively. Convallatoxin is the more potent substance in both tests, and it will be interesting to learn if this is due to a difference in the aglycone moiety or in the sugar residue. Cymarin contains a 2-desoxy sugar and the glycosidic linkage is susceptible to ready hydrolytic cleavage, while convallatoxin contains a firmly bound hexose unit.

According to W. Voss and G. Vogt, *Ber.*, 69, 2333 (1936), convallatoxin probably is the only glycoside of cardiac activity present in *Convallaria majalis*, but it is accompanied by other glycosidic substances. The crude extract has hemolytic and soap-forming properties probably due to the presence of an imperfectly characterized saponin known as convallarin. From the commercial preparation Convallamarin-Merck, Voss and Vogt isolated in a nearly pure but non-crystalline condition a glycoside to which they gave the name **convallamarin**, $C_{44}H_{70}O_{19} \cdot 3H_2O$. The purification was accomplished by a combination of distribution, adsorption, and fractional precipitation procedures. The material had no more cardiac activity in the frog test than could be accounted for by the presence of a trace of convallatoxin, and very feeble hemolytic properties were attributed to a slight amount of convallarin. Convallamarin does not give the Legal test and is not precipitated by cholesterol; the presence of one double bond was indicated by hydrogenation.

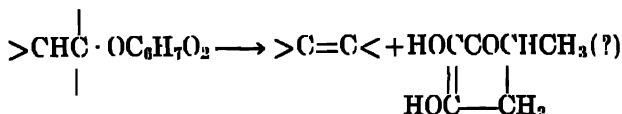
The glycosidic part of convallamarin is composed of two rhamnose and one glucose units and hydrolysis with aqueous acids proceeds no better than in the case of cardiac glycosides which likewise have a hydroxyl or methoxyl group in the sugar unit adjacent to the glycosidic link (rather than CH_3). Under ordinary conditions hydrolysis is only partial and the products are somewhat altered during the drastic treatment required. Voss and Vogt, noting in simple cases that the alcoholysis of a glycosidic linkage proceeds far more readily than hydrolysis, discovered an excellent method of cleavage which should prove of great value in other cases. This consisted in allowing a solution of convallamarin in 2% methyl alcoholic hydrochloric acid to stand for eight days at 35°. The sugar units were eliminated quantitatively as methyl glycosides and the aglycone was isolated in excellent yield in unaltered condition. The aglycone, **convallamaretin**, is assigned the probable formula $C_{26}H_{40}O_5$. It is not a lactone, it contains one double bond, and the Zerewitinoff determination, even at high temperatures, reveals the presence of only two hydroxyl groups.

Antiarins (addition to p. 299). Two isomeric glycosides have been isolated from the gum resin of the upas tree, *Antiaris toxicaria*, a material once used as an arrow poison. One form, now known as α -antiarin, was isolated by Mulder as early as 1838, and the β -isomer (m.p. 225°)

was discovered later by Kiliani. Both varieties of antiarin yield the same ring compound later by hydrolysis; they differ in that the β -form yields rhamnose while the α -form yields an isomer of rhamnose. In a reinvestigation of Kiliani's samples, Tschesche and Haupt, *Ber.*, 69, 1377 (1936), found by analysis and lactone titration that the antiarins have the composition $C_{29}H_{42}O_{11}$. The aglycone resulting from the hydrolysis of the glycosides was characterized as dianhydroantiarigenin, $C_{23}H_{38}O_6$, m.p. 165-167°. This substance shows no characteristic absorption in the region 230-280 $m\mu$, and consequently the anhydro linkages are not conjugated with one another or with a carbonyl group which Kiliani had recognized as present in the glycosides and in the aglycone. Although β -antiarin does not respond to sensitive tests for aldehydes, Tschesche and Haupt note that the same is true of strophanthidin. As the dianhydro compound contains only one secondary hydroxyl group, these investigators call attention to the possibility that antiarigenin, $C_{23}H_{32}O_7$, may differ from strophanthidin only in the presence of an additional tertiary hydroxyl group (probably at C_6).

K. K. Chen, A. L. Chen and R. C. Anderson (*loc. cit.*, p. 417, private communication from Dr. K. K. Chen) report the following activities for the two glycosides: α -antiarin, 0.13 mg. (cat unit), 0.00050 mg. (frog dose); β -antiarin, 0.10 mg. (cat unit), 0.00039 mg. (frog dose). The somewhat greater activity of β -antiarin in both tests shows that the nature of the sugar moiety is of definite, if minor, significance.

Calotropin (addition to p. 299). Hesse and Reicheneder, *Ann.*, **526**, 252 (1936), established for this African arrow poison the probable formula $C_{28}H_{40}O_9$. On being heated at 230° in high vacuum, the substance is cleaved into calotropagenin ($C_{23}H_{32}O_6$), an isomer of strophanthidin, and a methylreductinic acid, probably as follows:



The methylreductinic acid, which in the free state has powerful reducing properties, probably is linked to the genin through one of the enolic hydroxyl groups, as calotropin is stable in the air.

Basic Constituents of the Secretions (p. 303). In a further investigation, Jensen and K. K. Chen, *J. Biol. Chem.*, 116, 87 (1936), found that the "bufotenines" of different origins⁹³ are identical with the bufotenine investigated by Wieland and co-workers.

Bufotalin (pp. 306-311). In a further investigation of this genin and its companion substances, Wieland, Hesse and Hüttel, *Ann.*, **524**, 203 (1936), developed a simpler and improved method of isolation based upon the use of chromatographic adsorption. The material was extracted with

chloroform from the dried secretion, de-fatted with petroleum ether, and adsorbed on alumina from an acetone solution, a yellow impurity serving as an indicator for bufotalin. After developing the chromatogram with chloroform, bufotalin and a mixture of companion substances were found in different zones and a fairly sharp separation was possible. From 33,000 toads there was obtained 36 g. of bufotalin and about 29 g. of companion poisons, or about 2 mg. of active material per toad.

It was found that bufotalin has the same type of absorption spectrum as scillaridin A (p. 299), the maximum in each case being at 300 m μ . Since there is a considerable body of evidence indicating that the absorbing group of scillaridin A is a doubly unsaturated lactone ring, it seemed likely that the same group is present in bufotalin. This inference was strengthened by the observation that the genin was not attacked by perbenzoic acid and that on ozonization of acetyl bufotalin a substance giving a color test for glyoxylic acid was formed, and consequently Wieland is inclined to believe that both of the double linkages of bufotalin are located in the lactone ring and that the nucleus is saturated. This view would necessitate the rejection of the previous conception of the relationship between bufotalin and bufotoxin (pp. 304-305), and, if the suberylarginine residue is eliminated by a process of true hydrolysis, it would have to be supposed that bufotoxin contains one molecule of water of crystallization not eliminated in drying: $C_{26}H_{37}O_7 \cdot OCOR \cdot H_2O$, rather than $C_{26}H_{37}O_6 \cdot OCOR$. Wieland favors this view and suggests that the suberylarginine residue is attached to the tertiary hydroxyl at C₁₄. A further difficulty is encountered in attempting to account for the relationship of bufotalien to bufotalin and to bufotoxin (p. 310). Since the two additional nuclear double linkages of bufotalien produce no noticeable effect on the absorption spectrum, Wieland thinks it is unlikely that they are conjugated with one another, but if this is the case it is difficult to see why they invariably appear concurrently in the dehydration reactions. The acetoxyl group which is susceptible to ready elimination along with the tertiary hydroxyl at C₁₄ can hardly be present in secondary combination if an activating double bond actually is absent, and Wieland considers that it must be located at C₃ or C₆. In either case the dianhydro compound might contain unconjugated double bonds, but it is odd that there should be no differentiation in the ease with which they are introduced. It is evident that there are inconsistencies in the interpretation suggested on the basis of the absorption spectra. Bufotoxin itself has the same type of absorption spectrum as bufotalin and bufotalien, and it is perhaps significant that the relationship between the conjugated compound and the dianhydro genin of the series is exactly the same as that between scillaren A and scillaridin A (p. 299). In the

latter case it seems very odd that both compounds exhibit similar absorption maxima, for there are definite indications that the dianhydro compound contains a conjugated system of double bonds in the nucleus (p. 298) and yet there is no evidence of selective absorption at $240\text{ m}\mu$ or at $280\text{ m}\mu$, as would be expected if conjugated double bonds were present in different rings, or in the same ring, respectively. Both problems probably are related, and both remain obscure.

In the course of their work, Wieland, Hesse and Hüttel made a further characterization of two companion substances observed in the earlier investigation of Wieland and Hesse.¹ These substances are acetyl-free and may be related to the hypothetical genin $\text{C}_{24}\text{H}_{34}\text{O}_6$ of which bufotalin is the acetyl derivative. **Bufotalidin**, $\text{C}_{24}\text{H}_{32}\text{O}_6$, m.p. 228° , contains one more double bond and one more hydroxyl group than bufotalin. The lactone ring is opened by methyl alcoholic potassium hydroxide and there is formed an oxidic ester exactly analogous to isoscillaridinic acid methyl ester (VIII, p. 297). Coupled with the fact that the genin shows an absorption maximum of $300\text{ m}\mu$, this observation clearly points to the presence of the doubly unsaturated lactone ring characteristic of scillaridin A. **Bufotalinin**, $\text{C}_{24}\text{H}_{30}\text{O}_6$, also shows an absorption maximum at $300\text{ m}\mu$. In the Zerevitinoff test for active hydrogen, two moles of gas were liberated at 28° and a further mole was evolved at 95° , suggesting the presence of two secondary and one tertiary hydroxyl groups. Treatment with methyl alcoholic potassium hydroxide gave a yellow salt of a stable enol ester. That an oxidic ester is not formed may mean that the usual C_{14} -hydroxyl group is absent or that the steric arrangement does not permit cyclization.

Other Genins (pp. 311-316). Wieland, Hesse and Hüttel (*loc. cit.*) found that the absorption spectra of gamabufogenin and arenobufagin are similar to those of scillaridin A and bufotalin, showing maxima in the region $300\text{ m}\mu$. This is a preliminary indication of the presence in these toad poisons of the type of lactone ring characteristic of scillaridin A.

Cinobufagin (p. 312). X-ray crystallographic determinations of the molecular weights of cinobufagin, its acetyl derivative, and of cinobufagone have confirmed the previous indications that cinobufagin has the formula $\text{C}_{28}\text{H}_{34}\text{O}_6$ [Crowfoot and Jensen, *J. Am. Chem. Soc.*, **58**, 2018 (1936)]. Tschesche and Offe, *Ber.*, **69**, 2361 (1936), found that the absorption spectrum is similar to those of scillaridin A and bufotalin (p. 420), having a maximum at about $290\text{ m}\mu$. On ozonization of the genin they detected the formation of formic acid, and the presence of a free or latent aldehydic group was established in the product of careful hydrolysis. These observations strongly suggest the presence of the

doubly unsaturated lactone ring characteristic of scillaridin A. As Tschesche and Offe observed the formation of a hexahydro compound (two forms) on hydrogenation, while Jensen⁷ obtained a tetrahydro derivative, it is probable that the genin has one nuclear double bond which is somewhat inert, in addition to two double linkages in the lactone ring. On saponification of the hexahydro compounds, the acids formed promptly undergo dehydration without loss of the acidic character, and from this Tschesche and Offe inferred that the genin has a tertiary hydroxyl group at the 14-position which enters into the formation of an oxide ring. There are as yet no indications concerning the location of the secondary hydroxyl group, the nuclear double bond, and the acetoxyl group.

Bufagin (pp. 314-315). Tschesche and Offe, *Ber.*, 69, 2361 (1936), found that bufagin ("marinobufagin") has an absorption maximum at 300 m μ and absorbs three moles of hydrogen on catalytic hydrogenation. Two of the double bonds probably are located in the lactone ring and, since Jensen and Evans¹⁴ obtained a tetrahydro derivative, the third double bond seems to be fairly inert and may be situated in the nucleus (possibly at C₉-C₁₁). Jensen (private communication) has suggested that the group at C₁₀ is CH₂OH rather than CHO, as proposed in the first edition, and if this is the case the genin probably contains two tertiary hydroxyl groups (possibly at C₇ and C₁₄).

Chapter VII

Triterpenoid Sapogenins (pp. 318-321). The investigation of the structures of compounds of this group continues to attract considerable interest, but the problem is not yet solved and the views of prominent workers in the field are still speculative and provisional. Ruzicka has abandoned the skeletal structure previously attributed to ring A of the triterpenoid compounds (I, p. 320) on the basis of dehydrogenation experiments [Ruzicka, K. Hofmann and Frei, *Helv. Chim. Acta*, 19, 386 (1936)]. The trimethylnaphthol isolated in some of the experiments was believed to be a 1,2,7-trimethylnaphthol because it yielded a hydrocarbon at first regarded as sapotalene on treatment with zinc dust at 400°, but the substance was not identical with any of the five possible hydroxyl derivatives of sapotalene, all of which were synthesized for comparison [Ruzicka, Hösli and K. Hofmann, *ibid.*, 19, 370 (1936)]. Suspecting that a rearrangement occurs in the zinc dust distillation, and in order to avoid this complication, the trimethylnaphthol was converted into a perhydro-

genated hydrocarbon and this was dehydrogenated. The product was at first believed to be 1,2,8-trimethylnaphthalene, but in later work it was identified as 1,2,5-trimethylnaphthalene [Ruzicka, K. Hofmann and Schellenberger, *ibid.*, 19, 1391 (1936)]. The hydroxy compound obtained by dehydrogenation was found to be identical with synthetic 1,2,5-trimethyl-6-naphthol. To account for the formation of this substance, Ruzicka altered his proposed formula, for example for oleanolic acid (IV, p. 321), by retaining the hydroxyl group at position 2, transposing the *gem*-dimethyl group to 1, and moving the angular methyl group to the bridge head adjacent to position 4.

Further experiments on the degradation of oleanolic acid and sumarensolic acid have led Ruzicka to alter also the formulation of ring E [Ruzicka, Hösli and K. Hofmann, *ibid.*, 19, 109 (1936); Ruzicka and K. Hofmann, *ibid.*, 19, 114 (1936)]. The carboxyl group is transposed to the alternate bridge head and the double bond is moved to the adjacent γ,δ -position in the ring. The properties of betulin can be interpreted satisfactorily on this basis [Ruzicka and Isler, *ibid.*, 19, 506 (1936)]. Gypsogenin has been related to the most extensively characterized compounds of the group. Ruzicka and Giacomello, *ibid.*, 19, 1136 (1936), converted this substance into oleanolic acid on reduction of the carbonyl group by the Wolff-Kishner method.

In an attempt to identify the picene homologue obtained as a dehydrogenation product, Ruzicka and Mörgeli, *ibid.*, 19, 377 (1936), synthesized the 3,8-dimethyl and the 3,9,10-trimethyl derivatives of picene, but neither substance was identical with the degradation product. Drake and Duvall, *J. Am. Chem. Soc.*, 58, 1687 (1936), obtained sapotalene, 2,7-dimethylnaphthalene, and a picene homologue from ursolic acid by selenium dehydrogenation.

From his studies of the degradation of oleanolic acid and hederagenin, Kitasato, *Acta Phytochim. (Japan)*, 9, 43, 61, 75 (1936), has arrived at a provisional conception of the structures which is somewhat different from his earlier formulation (V, p. 321) but very similar to that most recently suggested by Ruzicka. His formulation of ring E coincides with that of Ruzicka except that the double bond is placed at the alternate γ,δ -position; in ring A the hydroxyl and *gem*-dimethyl groups are assigned the positions 2 and 1, respectively, as above. A hydroxyl group in this position of the phenanthrene ring system (ABC) would correspond to the characteristic C₃-hydroxyl group of the sterols. According to Askew, *J. Chem. Soc.*, 1585 (1936), the results of surface film measurements are in accord with this formulation [see also Spring, *Chemistry and Industry*, 55, 964, 1050 (1936)].

Other work on the triterpenoid sapogenins is reported in the following

papers: Ruhkopf and Mohs, *Ber.*, **69**, 1522 (1936); Grasshof and Wedekind, *ibid.*, **69**, 2686 (1936); Sone, *Acta Phytochim. (Japan)*, **9**, 83 (1936); Kuwada, *J. Pharm. Soc. Japan*, **55**, 1258 (1935); **56**, 469 (1936); Fujii and Matsukawa, *ibid.*, **55**, 1322 (1935); **56**, 408 (1936); Nozoe and Katsura, *J. Chem. Soc. Japan*, **57**, 692 (1936); Ruzicka and Leuenberger, *Helv. Chim. Acta*, **19**, 1402 (1936).

The substances friedelin and cerin, which can be extracted from cork with ethyl acetate, have been recognized as triterpenoid compounds by Drake and co-workers (Jacobsen, Shrader, Cumpbell, Haskins), *J. Am. Chem. Soc.*, **57**, 1570, 1854 (1935); **58**, 1681, 1684 (1936). Friedelin ($C_{30}H_{50}O$) is a ketone, cerin ($C_{40}H_{70}O_2$) is a hydroxyketone, and both compounds can be converted into the same hydrocarbon. The triterpenoid character was established by the observation that the alcohol from friedelin on dehydrogenation with selenium yields sapotalene, 1,2,8-trimethylphenanthrene, and a picene homologue. By oxidative degradation it was found that friedelin contains the group $-CH_2COCH<$.

Another compound apparently related to oleanolic acid and hederagenin is quinovic acid, which has been studied by Wieland and co-workers (Erlenbach, Hoshino, Utzino, K Kraus, A Hartmann, Dietrich), *Ann.*, **453**, 83 (1927); **479**, 179 (1930); **488**, 242 (1931); **497**, 140 (1932); **522**, 191 (1936). The compound is obtained by the hydrolysis of the glycoside quinovin, found in cinchona bark. Quinovic acid is a hydroxy dibasic acid, $C_{30}H_{46}O_7$. It loses carbon dioxide readily when heated and yields pyroquinovic acid. Dehydrogenation with selenium affords in unusually good yield a hydrocarbon ($C_{27}H_{40}$) which was characterized as a picene homologue by examination of the absorption spectrum. In addition to this evidence, the chemical properties of the compound indicate a relationship to the triterpenoid sapogenins.

Other additions to the large group of substances now classified as triterpenoid in character are the alcohols lanosterol and onocerin (p. 358, which previously were regarded as sterols.

New Saponins and Sapogenins (pp. 321-324). Tschesche, *Ber.*, **69**, 1665 (1936), isolated tigonin in a pure condition from *D. lanata* by precipitating the substance as the sparingly soluble and nicely crystalline molecular compound with cholesterol. Tigonin was recovered from purified cholesterol tigonide by dissociation with pyridine and precipitation with ether. The addition compound contains one molecule of each component. As the solubility in alcohol (0.15 g in 100 cc. of 95% alcohol at 18°) is considerably greater than that of cholesterol digitonide (0.014 g.), tigonin is less suitable than digitonin for use as a precipitant. Tschesche noted that, since the sugar residue of tigonin can be linked to the aglycone nucleus only at the C_3 -hydroxyl group, this probably

represents the point of attachment of the glycoside units of the other saponins. The plant heart poisons are now recognized as being similarly constituted in this respect.

From the California soap plant *Chlorogalum pomeridianum*, Jurs and Noller, *J. Am. Chem. Soc.*, 58, 1251 (1936), isolated a crystalline saponin to which they assigned the name amolonin (see table, p. 324). The aglycone obtained on hydrolysis is identical with that from tigonin. Smilagenin, a substance which appears to be an isomer of sarsasapogenin and which is more soluble than this substance, was isolated from Jamaican sarsaparilla root (*Smilax ornata*) by Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936). Smilagenin is precipitated slowly by digitonin and yields a desoxy compound different from desoxysarsasapogenin.

Structure of Digitogenin and Gitogenin (pp. 323-336). At the time of the publication of the first edition it was not known with certainty whether the hydroxyl groups in ring A of digitogenin and gitogenin are situated at the 2- and 3-positions or at positions 3 and 4. The evidence from the early literature on this point was in some respects definitely contradictory, as certain of the observations indicated the 2,3-structure while other data were reconcilable only with the alternate formulation (pp. 325-327, 335-336). The most important arguments in favor of placing the hydroxyl groups at the 3- and 4-positions centered around the composition and properties of the "acid A" of Windaus and Willerding (pp. 326, 334). While the alternate formulation afforded a much more reasonable interpretation of many of the reactions of the genins, it seemed to be definitely excluded on the basis of the properties attributed to acid A by Windaus and Willerding. Recognizing the importance of evaluating this evidence, Tschesche and Hagedorn, *Ber.*, 69, 797 (1936), reinvestigated the acid and its derivatives. They found the early work to be seriously in error. Acid A is not $C_{26}H_{38}O_{12}$, as it was at first supposed, or $C_{27}H_{40}O_{12}$, according to the revised formulas of the genins, but $C_{21}H_{32}O_{10} \cdot H_2O$. The acid is tetrabasic and not pentabasic and it is not a compound of the malonic acid type but a β -keto acid. The early analyses of the decarboxylation product, "acid B," and of its ester also were found to be erroneous. Acid B is a tribasic acid of the composition $C_{22}H_{32}O_8 \cdot H_2O$, and the ester does not liberate methane in the Zerewitinoff test. All of the arguments based upon the previous reports clearly fall into the discard, and the balance of evidence now indicates almost beyond question that the hydroxyl groups in the terminal ring of digitogenin and gitogenin are located at C_2 and C_3 .

On the basis of the revised formulas for acids A and B and the new evidence concerning the nature of these substances, Tschesche and Hagedorn suggested formulations consistent with the 2,3-structure which

provide an entirely rational account of the observations. The inconsistencies in the alternate interpretation of other relationships also vanish. Digitogenic acid no longer need be regarded as a β -keto acid of unique properties, but can be assigned the more rational formula of a γ -keto acid (V, p. 326). The formation of an enol lactone from the trimethyl ester of oxodigitogenic acid (p. 335) becomes understandable, and the opening of ring A in the oxidation (p. 336) follows the course expected in analogy with other cases. The only observation with which the revised formulas do not appear to be in harmony is the difference in the velocity of hydrolysis of the two ester groups of gitogenic ester noted by Jacobs and Simpson²⁷ (p. 327). It seems necessary to conclude that such evidence does not provide a reliable guide in the determination of structure.

Since acids A and B do not have the formulas or the properties originally attributed to them, the reasoning leading to the proposal of an ethylene oxide structure for the sapogenin side chain (p. 334) is no longer valid. The suggestion, however, still merits consideration and further evidence will be required in order to distinguish between the alternate formulations for the terminal part of the side chain.

Determination of Empirical Formulas by Analysis (addition to p. 325). Frequent reference has been made to the difficulty in determining the formulas of natural products by direct analysis (pp. 133, 291, 295, 325), and to the difficulties often encountered in the combustion of phenanthrene derivatives (pp. 54, 206, 225). Ordinary carbon-hydrogen determinations are not sufficiently precise to distinguish between homologues of molecular weights of the order of 350-450, where the differences in the composition for alternate formulas is no more than 0.06-0.5% carbon and 0.09-0.2% hydrogen, depending upon the amount of oxygen present in the compound. In some cases it is possible to analyze derivatives having higher percentages of oxygen, halogen, or nitrogen than the parent substance, for this may increase the spread in the carbon values for alternate formulas differing by CH_2 to as much as 0.4-0.6%, although the hydrogen values are but little affected. Reinitzer, *Monatsh.*, 9, 421 (1888), characterized cholesterol by the analysis of the acetate dibromide, Windaus, v. Werder and Gschaidler, *Ber.*, 65, 1006 (1932), made use of sterol bromoacetates and nitrobenzoates, and Drake and Jacobsen, *J. Am. Chem. Soc.*, 57, 1570 (1935), employed *p*-iodobenzoyl derivatives.

Naturally occurring acids and lactones often can be characterized satisfactorily by a careful determination of the neutralization equivalent [Windaus and Brunken, *Z. physiol. Chem.*, 140, 48 (1924); Stoll, A. Hofmann and Peyer, *Helv. Chim. Acta*, 18, 1247 (1935)]. In the case of

an alcohol, a determination can be made of the saponification equivalent of the acetate or benzoate [Vesterberg, *Ark. Kemi, Mineral. Geol.*, 9, No. 27, 1 (1926); Sandqvist and Gorton, *Ber.*, 63, 1935 (1930); 64, 2167 (1931); Windaus, v. Werder and Gschaidler, *loc. cit.*]. Drake and Jacobsen (*loc. cit.*) determined the saponification equivalents of a series of homologous esters. In any of the methods depending upon acidimetry, it usually is necessary to employ a rather large sample (0.5-1 g.) in order to determine the molecular weight with an accuracy of 2-3 units (Stoll, Sandqvist).

The X-ray and crystallographic method also is of distinct value in the determination of molecular weights in those cases where it is applicable (pp. 308, 312, 314, 421). All these methods have been found useful, and yet they all are subject to some limitations.

Recently Fieser and Jacobsen, *J. Am. Chem. Soc.*, 58, 943 (1936), applied to the problem the technique of precision combustion developed by Baxter and Hale, *ibid.*, 58, 510 (1936), in an investigation of the atomic weight of carbon. The combustion is conducted at a rather high temperature in a quartz tube with a filling of platinum catalyst and a fairly short layer of copper oxide. Typical results obtained in individual analyses with samples weighing about 1 g. are as follows (calculated for C=12.01).

	% C		% H	
	Found	Calcd.	Found	Calcd.
Triphenylbenzene ($C_{18}H_{14}$)	94.074	94.079	5.918	5.921
Dihydrocholesterol ($C_{27}H_{48}O$)	83.439	83.436	12.433	12.447
Chlorogenicin ($C_{17}H_{14}O_4$)	74.961	74.956	10.250	10.250
Sarsasapogenin ($C_{27}H_{42}O_4$)	77.827	77.835	10.644	10.644
Fichtelite ($C_{27}H_{42}$)	(86.875)	86.945	13.062	13.056

In general the results agreed with the theoretical values within 0.02% for carbon and 0.01% for hydrogen, and it is evident that in at least some cases substances of both plant and animal origin can be prepared in a very high state of purity. The percentages found for sarsasapogenin fully confirm the C_{27} -formula. The precision analysis of chlorogenicin led to a revision of the formula $C_{26}H_{42}O_4$, previously attributed²³ to the sapogenin on the basis of numerous macro- and micro-analyses of the compound and its derivatives, of saponification equivalents, and of molecular weight determinations.

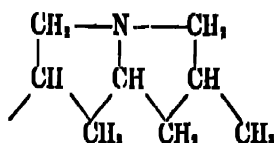
Sarsasapogenin (p. 336). From surface film measurements Askew, Farmer and Kon, *J. Chem. Soc.*, 1399 (1936), found for tigogenin, gitogenin, and sarsasapogenin the limiting areas 38, 39.5, and 42 sq. Å per molecule, respectively. The accepted formulas for the first two compounds are entirely consistent with the areas found. The value for

sarsasapogenin, however, cannot be reconciled with a formula in which the water-attracting hydroxyl group is at C₁₁, as suggested by Simpson and Jacobs, rather than at the end of the molecule. From an examination of models the English investigators conclude that the hydroxyl group can only be accommodated at C₂, C₃ or C₄. They note that the 4-position is unlikely because sarsasapogenin (but not its epimer) is precipitated by digitonin, in contrast to 4-cholestanol and its epimeric form. The fact that sarsasapogenone is not isomerized by fairly vigorous treatment with alkali also indicates that the carbonyl group probably is not at the α -position 4, although by analogy with the α -decalones this argument may be valid only in the *cis* (coprostane) series.

From the fragmentary information at present available, it appears likely that sarsasapogenin is a coprostane derivative hydroxylated in the usual sterol position (C₃), that is, that it has the same structure as tigogenin but differs in the manner in which rings A and B are joined. The dibasic lactone acid III would then have the formula II, p. 332 (erroneously attributed in the first edition to the oxidation product of tigogenic acid), while the lactone VII would be assigned the structure of V, p. 331, and the configuration of a coprostane, rather than a cholestane, derivative. (According to a preliminary report [*Chemistry and Industry*, 55, 925 (1936)] which has become available since the above was written, Kon and Farmer have degraded sarsasapogenin to actiobilanic acid. This result proves that the sapogenin belongs to the coprostane series and that one of the oxide rings is linked to C₁₆).

Alkaloids Related to the Sapogenins (addition to p. 337). There are indications that certain alkaloids and alkaloidal aglycones contain the reduced cyclopentenophenanthrene ring system and are in fact closely related in structure to the steroid sapogenins. The eyes or shoots of potatoes contain the glycoalkaloid **solanine**, together with the corresponding aglycone, **solanidine**. The formula established for solanidine, C₂₇H₄₃ON, is suggestive of a relationship to the sapogenins of the digitalis group or to cholesterol, and like the latter compound solanidine contains one double bond and is precipitated by digitonin. Soltys and Wallenfels, *Ber.*, 69, 811 (1936), defined these relationships more clearly than in the earlier literature and, in order to extend the evidence, they submitted to dehydrogenation with selenium the anhydro derivative of solanidine, known as solanthrene or solanidiene. From the reaction mixture they isolated a substance which was fully identified as the Diels hydrocarbon. In analogy with the many known cases, this establishes the nature of four of the six rings indicated by the analysis and properties. Soltys and Wallenfels noted that the precipitability with digitonin affords some indication that the hydroxyl group is located in the 3-posi-

tion, and they suggested that the double bond may occupy a position corresponding to that of cholesterol. Clemo, W. M. Morgan and Raper, *J. Chem. Soc.*, 1299 (1936), suggested a plausible if entirely provisional formulation which incorporates the nitrogen atom into the cholesterol side chain. Their formula represents solanidine as differing from cholesterol only in having the side chain:



From the leaves of the South African winter cherry, *Solanum pseudo-capsicum*, Barger and Frankel-Conrat, *ibid.*, 1537 (1936), isolated the alkaloids solanocapsine and solanocapsidine. The provisional formulas are $\text{C}_{26}\text{H}_{44}\text{O}_2\text{N}_2$ and $\text{C}_{26}\text{H}_{42}\text{O}_4\text{N}_2$, respectively. The second of these substances on dehydrogenation with selenium yielded the Diels hydrocarbon.

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